University of Alberta

Pharmacokinetic and Pharmacodynamic Evaluation of Acebutolol

By

Seyed Abolfazl Mostafavi Isfahani ©

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ”***Pharmacokinetic and Pharmacodynamic Evaluation of Acebutolol***” submitted by Seyed Abolfazl Mostafavi in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Pharmacokinetics).

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ABSTRACT

Beta-blockers are recommended as first line therapy for treatment of hypertension. Acebutolol (AC) is a β-blocker which has some degree of intrinsic sympathomimetic activity (ISA). It is metabolized by the liver, which suggests that any change in hepatic blood flow may alter its kinetics. Blood flow can be altered by any change in cardiac output (CO). Furthermore, the literature suggests that the CO is depressed less by β-blockers possessing ISA than by those without ISA. It was therefore proposed to study in detail the pharmacokinetics and the pharmacodynamics of AC in comparison with a β-blocker which is devoid of ISA (i.e. metoprolol, MET) to assess the effect of ISA in this regard.

The rat was chosen as the animal model. The jugular vein was cannulated for blood sampling and drugs were measured by a stereospecific HPLC method. The rat appears to be a good animal model for these studies, as the disposition kinetic of AC and MET enantiomers in this species were comparable to human. The disposition of AC was stereoselective and its enantiomers follow linear pharmacokinetics in the rat after iv administration of racemic doses of 5 to 50 mg kg^{-1}. Furthermore, it was found that the pharmacokinetics of AC enantiomers at steady state could be predicted by using the single dose data only after iv administration as AC accumulates after multiple oral dosing. Similarly, the plasma concentrations of MET enantiomers at steady state were also found to be greater than predicted by the single dose data after oral administration of racemate.

The interesting point observed after oral administration of AC was the existence of two peaks in the absorptive phase of our rats. This was not due to the influence of gastric pH, variable gastric emptying, enterohepatic recycling or formation of bile salt micelles. It was determined that site dependent absorption is the underlying mechanism for the double peaks observed after oral administration of AC.

With regard to a change in CO and regional blood flow (RBF), it was shown that AC and MET significantly reduced the CO and RBF in most organs after acute administration, as compared with the baseline values. These values, however, returned to normal after chronic administration of either AC or MET. Thus, our results suggest that the additional property of ISA possessed by AC did not induce statistically significant hemodynamic differences when compared to MET. This may reflect an insufficient degree of ISA possessed by AC.

Although there were no significant differences in pharmacokinetics or pharmacodynamics of AC as compared with metoprolol, a trend towards differences in these values was observed. The importance of hemodynamic effects of ISA should therefore not be dismissed when evaluating β-blockers that have a marked degree of ISA.

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Dedication

To my parents for their best
efforts to raise me.
To my wife for her unending
encouragement, support and patience.
To my children Hajar,
Mohammad and Mojtaba for
their patience and to their
bright future.

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GLOSSARY OF ABREVIATIONS

AC	acebutolol
ANOVA	analysis of variance
AUC	area under the plasma concentration-time curve
β	elimination rate constant
CL/F	oral clearance
C_{max}	peak concentration
Cl_{nr}	non-renal clearance
CL_R	renal clearance
CL_T	total clearance (systemic clearance)
° C	degree Celsius
CO	cardiac output
D	dose
DC	diacetolol
F	absolute bioavailability
g	gram(s)
h	hour(s)
HPLC	high-performance liquid chromatography
i.d.	inside diameter
ID	intra-duodenal
II	intra-ileal

IS	internal standard
ISA	intrinsic sympamimethic activity
ip	intraperitoneal
iv	intravenous
kg	kilogram(s)
L	liter(s)
µg	microgram(s)
µl	microliter(s)
mg	milligram(s)
min	minute(s)
ml	milliliter(s)
MRT	mean residence time
MSA	membrane stabilizing activity
NEIC	naphthylethylisocyanate
n	number of observations
ng	nanogram(s)
o.d.	outside diameter(s)
PO	per oral
RBF	regional blood flow
s	second(s)
SD	standard deviation

SEM	standard error of the mean
T_{max}	the time to peak concentration
$\sum X_U$	cumulative urinary excretion
τ	dosing interval
$t_{1/2}$	half-life
U	unit(s)
V_d	volume of distribution
V_d/F	volume of distribution after oral doses
V_{dss}	volume of distribution at steady state
vs.	versus

# ✷CHAPTER 1✷

INTRODUCTION

Beta Blockers and Hypertension

Kaplan stated that "hypertension has become the most frequent reason for visits to physicians as well as the leading indication for prescription drugs" (1). In the treatment of hypertension, substances blocking beta-receptors are among the most frequently used drugs, both in monotherapy and in combination with other agents. In addition they are widely used in other common clinical conditions such as supraventricular tachycardia, hyperthyroidism and anxiety neurosis. The success of these drugs resulted in the appearance of a large number of β-blockers in clinical practice, and still more are under development. When treating hypertension, however, physicians are faced with a variety of β-blockers with different ancillary properties to choose from. The properties differentiating beta-blockers include lipid solubility, cardioselectivity, intrinsic sympathomimetic activity (or partial agonist activity) membrane stabilizing activity and pharmacokinetic properties (Table 1-1).

Lipid solubility

Lipid solubility refers to the extent to which a drug partitions between an organic solvent and an aqueous buffer. This solubility is dependent upon the buffer used, its pH and on the temperature at which partitioning is measured. The relative degree of lipid solubility is an important factor in determining the rate of absorption from the gastrointestinal tract, plasma protein binding, distribution in the body, and elimination (2-4). In fact, for the hydrophilic drugs, all of the drug absorbed is bioavailable. In contrast, the bioavailability of some of the more lipophilic drugs may be little more than 10-40% despite the fact that these drugs are almost completely absorbed (3). In general, the lipophilic drugs are extensively metabolized during their first passage through the gut wall and liver and they show more plasma concentration variability. Consequently, the lipophilic β-adrenoceptor antagonists are eliminated rapidly with short half lives (Table 1-1). The β-blockers without first pass, however, have much longer elimination half lives and they demonstrate less plasma concentration variability (2).

There appears to be some relationship between the protein binding and the degree of lipophilicity. Lipophilic β-adrenoceptor blocking drugs appear to be more highly bound to plasma proteins than water soluble drugs, although there are some exceptions to this rule (5). Consequently, the magnitude of the volume of distribution exceeds the body volume for most lipophilic drugs indicating that accumulation occurs in the tissues. Finally, the more lipid soluble drugs appear to gain access to the central nervous system more easily and more rapidly. It is, therefore, considered that lipid soluble drugs are more likely to cause centrally mediated side effects although there is some suggestion that central effects may also occur with the water-soluble drug (6).

Table 1-1. Pharmacological and pharmacokinetic properties of some beta blockers. (adapted from references 7-10)

Drug	AC	AP	AT	CEL	ESM	MET	NAD	OX	PIN	PRO	STL	TIM
β-blockade Potency (propranolol = 1)	0.3	0.3	1	1	1	1	2-9	0.5-1	6	1	0.3	6
Relative β_1 selectivity	+	0	++	+	+ or ++	+ or ++	0	0	0	0	0	0
ISA	+	+	0	0	0	0	0	+ or ++	++	0	0	0
MSA	+	+	0	0	0	+	0	+	+	++	0	+
Bioavailability (%)	20-60	10	50-60	30-70	iv	40-50	30	25-60	100	30	60	75
First pass hepatic metabolism	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes	No	No
Elimination half life (h)	7-8	2-3	6-9	4-5	0.13	3-4	12-24	2	3-4	2-6	5-13	4-5
Protein binding (%)	20	85	<5	25	?	10-15	30	>90	57	>90	<1	60
[a]pK_a	9.7	9.7	9.6	9.7	?	9.7	9.7	9.5	8.8	9.5	9.8	8.8
[b]Relative lipid solubility	M	H	VL	M	L	M	VL	M	L	H	VL	M

a pK_a has determined in the pH at which the drug is 50% ionized. b Based on the partition coefficient octanol / water. Abbreviations : 0 = no activity; + = mild activity; ++ = moderate activity; +++ = marked activity, AC = Acebutolol, AP = Alprenolol, AT = Atenolol CEL = Celiprolol, ESM = Esmolol, MET = Metoprolol, NAD = Nadolol, OX = Oxprenolol, PIN = Pindolol, PRO = Propranolol, STL = Sotalol, TIM = Timolol; M = moderate, H = high, L = low, VL = very low,

Cardioselectivity

The β-adrenoceptor blockers may be classified as selective or nonselective, according to their relative abilities to antagonize the actions of sympathomimetic amines in some tissues at lower doses than those required in other tissues (11). β_1 selective blocking agents, when used in low doses, inhibit cardiac β_1 receptors resulting in bradycardia, a decreased contractile state and a lowered cardiac output. These agents however, have less influence on bronchial and vascular β_2 adrenoceptors (12,13). In higher doses, however, β_1 selective blocking agents will also block β_2 receptors.

Since β_1 blockers have less of an inhibitory effect on the β_2 receptors they have two theoretical advantages. The first is that these agents may be safer than nonselective blockers in patients with obstructive airway disease, as β_2 receptors remain available to mediate adrenergic bronchodilation (13-15). The second advantage is that β_1 selective blockers in low doses may not block the β_2 receptors that mediate dilation of systemic peripheral arterioles. Commonly used β_1-blockers include atenolol, metoprolol (MET) and acebutolol (AC, Table 1-1).

Membrane stabilizing activity

Certain β-blockers show a quinidine-like or "local anesthetic" effect on the cardiac action potential when they are used in concentrations well above therapeutic levels. This effect is called membrane stabilizing activity (MSA, 16,17). There is no evidence that MSA is responsible for any direct negative inotropic effects of β-blockers since both drugs with and without this property equally depress left ventricular function (15,17). Since MSA can be demonstrated only at doses 50 to 100 times higher than those necessary for effective β-blockade it is not considered to be clinically relevant (15). β-Blockers that show MSA include AC, MET and pindolol (Table 1-1).

Intrinsic sympathomimetic activity

Certain β-blockers cause a slight to moderate activation of the β-receptors, in addition to preventing access of natural or synthetic catecholamines to the receptor sites (17,18). This property which is shown by some β-blockers is called partial agonist activity or intrinsic sympathomimetic activity (ISA). β-blockers with or without ISA show different hemodynamic effects. It has been suggested that a β-blocker with ISA does not reduce the resting heart rate and cardiac output to the same extent as a β-blockers without ISA (19,20).

In terms of blood pressure, the mechanisms for the antihypertensive effects of β-blockers are different. β-blockade without ISA seems to decrease cardiac output (CO) as the primary action, while total peripheral resistance reflexly increases or remains unchanged. On the other hand, β-blockers with ISA lower blood pressure by reducing the peripheral resistance while maintaining CO (21,22). Accordingly, Man in't Veld and Schalekamp (21) have shown that the reduction in CO were also dependent on time and on the presence or absence of ISA. Furthermore, the reduction in CO is also dependent on the number of successive doses administered in addition to the relative degree of ISA. The greatest reduction of CO was observed in β-blockers without ISA in long term therapy, although this was minimal in those with the greatest degree of ISA (23). Interestingly, this study has shown that the response to various β-blockers is a reflection of the degree of ISA and not the presence or absence of β_1 selectivity.

Although β-blockers possessing ISA are the most physiologically attractive of this class of drugs the treatment of hypertension, the clinical relevance of these theoretical considerations must yet be confirmed. β-blockers that possess ISA include acebutolol, oxprenolol and pindolol (Table 1-1).

Acebutolol

History

AC is a racemic β-adrenergic receptor blocking agent discovered in 1968. Introduced in France in 1974, the product has since been used in Europe, Africa, Asia, South America, Canada and United States for the management of hypertension, ventricular arrhythmia and some other indications (24). AC is unique in possessing the ancillary pharmacologic properties of cardioselectivity, ISA and MSA (Table 1-1). It is effective in essential hypertension given once or twice daily as monotherapy or in combination with other antihypertensive agents and is as effective as other β-blockers (25,26).

Physicochemical properties

Chemical structures of racemic AC and DC are depicted in Figure 1-1. AC hydrochloride is a white, or slightly off-white crystalline, non-hygroscopic, practically odorless powder (27). The molecular weights of the base and hydrochloride salt are 336.43 and 372.89, respectively. AC has solubilities of 200 mg/ml in water and 70 mg/ml in alcohol at room temperature (27). The lipophilicity of AC is substantially less than that of labetolol or propranolol, but comparable to that of some other available β-blockers such as metoprolol (26). The apparent pK_a of the drug in water is 9.4 (Table 1-1).

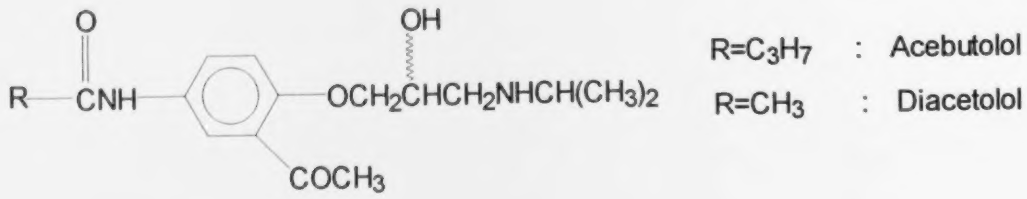

Figure 1-1. Chemical structures of racemic AC and its major metabolite DC.

Pharmacokinetics

Absorption

AC is rapidly and almost completely absorbed from the GI tract with peak plasma concentrations reached in 2-4 hours (28,29). The time to reach the C_{max} is similar for both enantiomers of AC in humans (30). The drug undergoes extensive first pass metabolism in the liver and the peak plasma concentration-time curve and AUC of AC is different among individuals, suggesting differences in first-pass liver metabolism (28-30). The absolute bioavailability of AC is about 40% in man (31). An increase in AUC and C_{max} of AC and DC in elderly patients has been reported as compared to healthy younger subjects (32), possibly as a result of decreased first pass metabolism. These values, however, are decreased by the presence of food (33). Further, the prolongation of half life for R- and S-enantiomers has been reported in elderly (34). Following single or multiple oral administration of AC, the plasma concentrations of DC are approximately 2-2.5 times higher than that of AC (28).

Distribution and protein binding

AC (11-19%) and DC (6-9%) are weakly bound to plasma proteins (35). The binding of drug to erythrocytes is, however, significant (36). The V_d of AC after dosing at 1 mg/kg was approximately 1.6 L/kg, which is one of the lowest of all β-blockers (37). The greater V_d has been reported for other β-blockers including metoprolol (5.5 L/kg) and propranolol (2.8-5.5 L/kg, 7). The apparent V_d appear to be decreased in elderly patients (32). After iv administration in rats, AC is distributed into many tissues including the heart, liver, kidneys, lungs, intestine, stomach, and the salivary glands (38). As AC has a low lipid solubility compared with other β-blockers its concentration in cerebrospinal fluid (CSF) is much less than that in plasma (39).

Elimination

The plasma concentrations of AC appear to decline in a biphasic manner. The half life of the initial distribution phase ($t_{1/2}\alpha$) is about 2 h and the elimination half life of 6-12 h has been reported in humans (28,30-32,36). Following a single oral dose of AC a half life of 7-11 h for DC has been reported. AC is eliminated from the body by liver metabolism as well as renal excretion (24,40).

Metabolism

Man, cat, dog, hamster, mouse, rabbit and rat qualitatively metabolize acebutolol in the same way, although there are some quantitative differences. The major human metabolite of AC is DC, which is pharmacologically active (24). AC is metabolized primarily by hydrolysis of its butyramide group, which forms a primary amine, acetolol. This metabolite then undergoes N-acetylation to produce its major metabolite DC (Fig 1-1). The concentration of DC is greater than that of its parent drug. Furthermore, the conversion of AC to DC in man occurs to a much greater extent after oral administration than after iv administration suggesting a "first pass effect". Although DC is a N-acetylated metabolite of AC, there is no correlation between a patient's acetylation status and the production of acetyl metabolite (41). There is no evidence that either AC or DC form glucuronide or sulfate conjugates in the liver (40).

Excretion

AC and its metabolites are excreted in urine, feces, and saliva. Following iv administration of AC, approximately 50% of the dose is excreted in urine within 1-4 days, about 30-40% as unchanged drug and 13-25% as diacetolol and 3% as acetolol. Approximately 33% is excreted in feces, 16% as AC and 17% as DC (24,40). The fraction of acetolol which is excreted in urine is negligible (~ 0.2%). As expected, decreased renal function results in decreases in the fraction of AC and DC excreted in urine. Following a single 200 mg oral dose of AC in patients with creatinine clearance of 14-56 ml/min, the fraction excreted in urine after 72 h as AC was about 6%. This value for DC was about 19% (36). The urinary excretion of AC, however, appears to be independent of urinary pH (42). Although the fraction of the dose which is excreted in urine is changed in renal failure, the half life of AC is not changed after renal failure as AC has other routes of elimination. The half life of DC, whose primary route of elimination is the kidneys, however, is markedly prolonged in renal failure. AC is also excreted through the saliva and is inversely related to saliva pH (39,43).

Pharmacodynamics

Beta blocking activity and selectivity

The β-blockade activity of AC has been demonstrated in humans and animals. AC inhibits tachycardia after iv administration of isoproterenol and also produces significant reduction in resting and exercise heart rates, with maximum β-blockade apparent at 3 h in healthy volunteers (44). AC also has a greater effect on cardiac β_1-receptors than on β_2-receptors (45). A long duration of action has been reported for orally administered AC in which the effect of DC with the long elimination half life should be considered (44). A linear correlation between log plasma concentration and degree of β-blockade has been shown (46,47). DC has also exhibited a significant cardiac β-blockade over a 24 h period. The data indicate that the cardioselectivity of DC is equal to, or even greater than that of AC (48).

Hemodynamic effect and ISA

Attempts have been made to find the significance of ISA possessed by some β-blockers. Hemodynamic studies of β-blockers with ISA show that these agents cause less reduction in heart rate and cardiac output after acute administration as compared with β-blockers without ISA (49). AC is the only β-blocker available that possesses both cardioselectivity and ISA (Table 1-1). Possession of ISA by acebutolol is well demonstrated (45,50). Furthermore, it has been shown that iv administration of AC and practolol, both with ISA, in contrast to propranolol, without ISA, had little effect on cardiac output and peripheral resistance (23).

Efficacy

AC is an effective antihypertensive agent which is administered once or twice daily (25,26,51). It is rapidly effective as a significant decrease in blood pressure two days after AC administration was observed, which correlated with reduction in plasma rennin activity (52,53). On comparison of AC with other antihypertensive drugs, AC used as a monotherapy is as effective, at rest, as diuretics (54) or methyldopa (55), and it is very effective in diuretic resistant hypertensive patients (56).

AC is as effective as other β-blockers (50), however, When compared with metoprolol, which is also cardioselective, metoprolol caused more bradycardia, reflecting the fact that AC possesses ISA (57). AC is also used for the treatment of ventricular and supraventricular arrhythmias as well as angina (40).

HPLC Assay

The stereosepecific assay for AC and its metabolite DC has been reported recently (ref). By using normal phase, the baseline separation of the peaks corresponding to AC and DC are attained (resolution, AC: R_s = 1.45; DC: R_s=1.50). The chromatograms are free from any interfering peaks and analysis of pure enantiomers of AC and DC did not reveal racemization.

There was an excellent linearity between the peak area rations (drug : internal standard) in plasma and urine ($r > 0.995$) over the concentration range examined. Typical calibration curves where Y is the peak area ration (R- or S-drug/internal standard) and X is the enantiomer concentration could be described for R and S-AC by $Y = 0.075 + 0.071(X)$ and $Y = 0.067 + 0.066(X)$ in plasma and $Y = -0.092 + 0.119(X)$ and $Y = -0.078 + 0.111(X)$ in urine, respectively. Typical calibration curves for DC in plasma can be described by $Y = 0.0085(X) + 0.0496$ and $Y = 0.0085(X) + 0.0427$ for the R- and S-enantiomers, respectively.

The observed assays interday and intraday coefficient of variation and error of measurements are shown in Tables 1-2 and 1-3 for AC and DC respectively. The extraction yields were of 91.1 ± 7.8 and 80.4 + 7.0 for 25 and 500 ng/ml respectively. In urine, yields were 80.8 + 3.85 and 87.4 + 7.19 for two and 100 mg/l, respectively. Extraction of R- and S-diacetolol from spiked samples of plasma were 26.0 ± 3.0 % at 50 ng/ml and 28.4 ± 3.4 % at 500 ng/ml. Recovery of DC was determined to be 30.1 ± 12.8 % at 0.5 mg/l and 29.0 ± 3.8% at 5.0 mg/L.

Table 1-2. Accuracy and precision-Acebutolol. Mean (SD)

Concentration (ng/ml)						
Added	Measured		Error %		Precision * %	
	R	S	R	S	R	S
10	10.4 (0.46)	10.4 (0.42)	3.5	4.4	4.5	4.0
25	25.3 (1.3)	25.0 (1.0)	1.1	0.1	4.9	1.0
50	51.9 (4.2)	51.4 (3.5)	3.8	2.8	8.0	6.9
100	101 (7.3)	100 (6.1)	1.7	0.3	7.1	6.0
200	204 (16)	202 (16)	2.1	1.4	7.8	7.9
500	499 (3.0)	499 (3.4)	-0.1	-0.1	0.70	0.67

* Coefficient of Variation %

Table 1-3. Accuracy and Precision-Diacetolol. Mean (SD)

Concentration (ng/ml)						
Added	Measured		Error %		Precision * %	
	R	S	R	S	R	S
10	10.9 (0.9)	10.9 (0.3)	9.2	9.7	8.2	2.6
25	24.8 (1.8)	25.2 (1.6)	-0.9	0.8	7.1	6.3
50	53.5 (3.9)	52.7 (5.0)	7.0	5.4	7.3	9.5
100	101 (6.7)	101 (6.7)	0.6	1.0	6.7	6.6
250	260 (11)	261 (13)	3.9	4.5	4.4	4.8
500	498 (3.6)	497 (4.4)	-0.5	-0.5	0.70	0.9

* Coefficient of Variation %

Rationale for the study of pharmacokinetics and pharmacodynamics of AC

Heart disease is one of the major causes of death in the world. In fact cardiovascular disease accounts for almost half of all deaths in each year and the costs associated with heart disease are staggering. It is a well recognized fact that hypertension is one of the contributing factors to the development of cardiovascular disease. There is a variety of medicinal agents from which to choose for hypertension treatment. The β-blockers are the first line therapy which is frequently selected. Although this class of antihypertensive drugs have several similarities, they lower blood pressure by different mechanisms which are linked to their ancillary properties such as affinity for β_1 and β_2 receptors, lipid solubility ISA and to their pharmacokinetic properties.

It is well established that properties of a drug may influence the intensity and duration of its own action or that of other drugs, by altering absorption, distribution or elimination. Such interactions in pharmacokinetic profiles may result from a variety of mechanisms including alterations in intestinal or urinary pH, plasma protein binding, drug metabolizing enzyme activity, and drug transport systems. In fact, by considering the important role played by the circulation in transporting the drugs through the body, it is reasonable to expect an alteration in disposition of a drug by hemodynamic changes induced by another. More specifically, it is now well established that hepatic blood flow can be an important determinant of the hepatic elimination of certain drugs, i.e., blood flow becomes progressively more rate limiting as the hepatic extraction ratios increase. The disposition of these drugs can be altered by drug-induced changes in liver blood flow.

As explained earlier, β-blockers are a class of antihypertensive drugs with different ancillary properties. In particular, β-blockers with ISA seem to induce a different hemodynamic response than those devoid of this property in a way that they cause minor reductions of heart rate and cardiac output. A reduction in cardiac output may reflect a proportional decrease in blood flow through the specific organ (i.e., liver). Thus it is plausible to see different pharmacokinetic profiles for highly extracted drugs when they are administered with β-blockers that possess ISA as compared to administration with those which are devoid of ISA. Therefore, a change in liver blood flow of highly cleared β-blockers will not only affect the clearance of other drugs but will affect their own clearance.

AC and MET are β-blockers with almost similar properties except the first one possesses ISA and the latter is devoid of ISA. It was therefore proposed to study in detail the pharmacokinetics and selected pharmacodynamics of these β-blockers to assess the presence of ISA in this regard.

Hypotheses

1. Cardiac output and regional blood flow (i.e. hepatic blood flow) may be altered by administration of a β-blocker (i.e. AC or MET).
2. Alteration of regional blood flow might be dependent on the degree of ISA possessed by some of β blockers (i.e. AC).
3. β-blockers with ISA (i.e., AC) may show less time dependent kinetics than those without this property.

Specific objectives and their rationale

Study the pharmacokinetics of AC and DC enantiomers in an animal model

Delineate the pharmacokinetics of AC enantiomers after administration of different doses of racemate to rats.

AC is administered as a racemate, although its beta-blocking activity resides mainly with the S-enantiomer (58). As enantiomers of racemic compounds often exhibit different pharmacokinetics, it is necessary to understand the time course of each enantiomer separately. Moreover, little has actually been demonstrated regarding the pharmacokinetics and dose ranging comparisons of AC enantiomers experimentally. Therefore it was difficult to choose suitable doses for further studies.

Further, investigations of the relationship between the plasma concentration and pharmacodynamic effects of AC, require that the pharmacokinetics of the drug be clearly delineated in a suitable animal model.

In this study, we investigate the pharmacokinetic characteristics of a dosing range comparison for AC and DC in male Sprague-Dawley rats.

Determine the pharmacokinetics of AC enantiomers after oral administration of racemate in rat

The influence of hepatic blood flow on hepatic drug elimination is dependent on the efficiency of the hepatic extraction ratio. However, virtually nothing is known about the extraction ratio of AC in the rat. Furthermore, for studying AC pharmacodynamics, it was necessary to know this value. We therefore studied the pharmacokinetics of AC enantiomers after oral administration of the racemate.

Detailed study of factors involved in the absorption profile of AC

The "erratic" plasma concentration-time profile of AC (59-62,33,34,36) and DC (62) were observed in the form of two concentration maxima after oral administration in humans and rats. Multiple peaking has been observed in the plasma concentration profile of several other drugs and it is attributed to enterohepatic recycling (63), discontinuous gastrointestinal absorption (64-66), formation of poorly absorbed micelle complex of drug with bile salts (67,68), variable gastric emptying (69,70), gastric pH (71), or reversible metabolism (72). Racemic AC was administered to different segments of rat intestine to determine if multiple peaks were due to discontinuous gastrointestinal absorption of AC. Furthermore, the possibilities of variable gastric emptying, formation of poorly absorbed micelle complex of drug with bile salts and reversible metabolism were investigated.

Delineate the pharmacokinetics of AC enantiomers over time

Although the concentration time profile of drugs after multiple dosing can be predicted after single dose administration, this is not achieved for drugs that show the time dependent kinetics. There are different reasons for the time dependent kinetics, including a change in cardiac output. As cardiac output is affected by some β-blockers, it is important to examine the possibility of time dependency for this class of drugs. Consequently the pharmacokinetics of AC enantiomers were studied in rats after multiple intravenous and oral doses.

Study the pharmacokinetics of MET enantiomers in rat

Delineate the pharmacokinetics of MET enantiomers after single and multiple dosing

As the purpose of our study was to compare the pharmacokinetics of AC, with ISA, and MET, without ISA, the dispositions of MET after single and multiple dose administration in rats were also investigated.

Study the pharmacodynamics of AC in rats

Determine the effect of AC on cardiac output and blood flow after single dose administration

Experimental data in humans and animals have shown that cardiac output is affected less by β-blockers possessing ISA. This implies that there may be a similar relationship with a change in blood flow. To study the effects of AC on regional blood flow, intravenous doses of AC were administered in jugular cannulated rats and the cardiac output and blood flow through different organs were measured by the radioactive microsphere method and compared with placebo.

Determine the effect of AC on cardiac output and blood flow over time

It is reported (23) that reduction in CO is dependent upon the number of successive doses administered and upon the relative degree of ISA present. Thus we sought to study the effect of AC after long term administration in rats to examine a change in regional blood flow.

Study the pharmacodynamics of MET in rats

Determine the effect of MET on cardiac output and blood flow after single dose administration

In parallel with the investigation on hemodynamic effect of AC we studied this effect with MET which is devoid of ISA in order to compare these two drugs in this regard.

Determine the effect of MET on cardiac output and blood flow over time

To examine the hypotheses that the hemodynamic effects of β-blockers are more pronounced after long term administration we investigated this effect after successive doses of MET in the rat model.

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✱CHAPTER 2✱

Pharmacokinetics Of Acebutolol Enantiomers After Intravenous Administration Of Racemate In a Rat Model: A Dosing Range Comparison*

INTRODUCTION

Acebutolol (AC) is a chiral β-adrenergic receptor blocking agent possessing ISA, membrane stabilizing effects and cardioselectivity (1). AC has been shown to be clinically effective in the treatment of hypertension, suppression of premature ventricular contractions and other cardiac arrhythmias (2,3). Like most β-blockers, the pharmacological activity of the racemate resides predominantly with the S enantiomer in man (4). In humans, AC is extensively metabolized upon first pass through the liver (5,6) and the disposition of both AC and its active metabolite diacetolol (DC) are stereoselective (6). Furthermore, the disposition of AC is reportedly stereoselective after oral administration of racemate in rat. The disposition of DC however, is not stereoselective in this species (7).

Information on the pharmacokinetics of AC has become more readily available during past years (8-19). Pharmacokinetic profiles of AC enantiomers however, may differ in different species (20). A preliminary study of rat and dog has shown that 6 h after oral administration of AC to rat and dog, the plasma concentrations of DC were

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only 12% of those of unchanged AC (5). In humans however, 2-3 times higher plasma concentrations for DC have been reported than those of unchanged AC (5,11).

In general, the use of AC in hypertension, angina pectoris or arrhythmias requires different doses. This may result in differing pharmacokinetic profiles of AC and DC enantiomers as it has been suggested that the pharmacokinetic of AC is saturable (11). In fact, Meffin *et al.* have shown that there is a more than proportional increase in AUC with increasing oral dose in man. Despite the reports in the literature, little has actually been demonstrated regarding the pharmacokinetics and dose ranging comparisons of AC enantiomers experimentally. Furthermore, investigations in to the relationship between the plasma concentration and pharmacodynamic effects of AC, require that the pharmacokinetics of the drug in a suitable animal model be clearly delineated.

In this study, we report the pharmacokinetic findings of a dosing range comparison for AC and DC in male Sprague-Dawley rats.

MATERIALS AND METHODS

Chemicals

Racemic AC and DC (as hydrochloride salts) were obtained from Rhone-Poulenc Rorer Canada Inc. (Montreal, Que, Canada). The internal standard (IS), racemic pindolol, was bought from Sigma (Illinois, USA). All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance

Male Sprague-Dawley rats (n = 6 in each group) weighing between 200-350 g were kept on a light-dark cycle of 12 hours at a room temperature of approximately 25° C. They were fasted for about 8 hours prior to, and for two hours following drug administration, with free access to water. Animals were catheterized with silastic tubing (0.025 in .i.d. × 0.037 in. o.d.; Dow Corning, Midland, MI, USA) at the right jugular vein while they were under general anesthesia with pentobarbital administered *via* the intraperitoneal route. The animals were allowed to recover overnight prior to the experiments. During this time the animals were individually stored in 18 in.× 9.5 in.× 8 in. polycarbonate rodent cages.

Dosing and sample collection

Rats were divided into four groups based on administered doses. One ml kg^{-1} of racemic AC dissolved in normal saline was administered as bolus doses of 5, 15, 30 and 50 mg kg^{-1} *via* the jugular vein in each group respectively. Blood (0.25 ml) was collected from the jugular vein cannula just prior to and at 2, 15, 30, and 45 min. and at 1, 1.5, 2, 2.5, 3.5, and 5.5 h after drug administration. Between each blood sample collection, 0.25 ml normal saline was administered *via* the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at -20° C until analyzed. Urine was collected and pooled for 24 h following drug administration. Urine samples were kept frozen at -20°C until just prior to analysis.

Stereospecific HPLC assay of AC and DC

Concentrations of R- and S-AC and R- and S-DC in plasma and urine were determined utilizing a previously reported stereospecific HPLC method (7,16,17). Briefly, samples were extracted with diethyl ether after addition of racemic pindolol as the IS and subsequent alkalization with 1 M sodium hydroxide. After the extract was evaporated, the resulting residues were derivatized with the homochiral reagent, S-(+)-1-(1-naphthyl)ethyl isocyanate. The resulting diastereomers corresponding to the enantiomers of AC and DC were chromatographed *via* normal phase HPLC using fluorescence detection. For urine sample analysis, specimens were diluted 1:10 (v/v) in HPLC grade water prior to extraction, derivatization, and subsequent analysis.

Pharmacokinetic data analysis

The plasma concentration-time data of AC enantiomers were fitted to bi-exponential functions. Nonlinear regression analysis was performed using version 4 of PCNONLIN computer software program (21). The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. The area from the last concentration point C_{last} to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points of the log linear terminal elimination phase of the plasma concentrations versus time curve. The terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\beta$. Total body clearance (Cl_T) was calculated as D/AUC, where D was the enantiomeric dose administered and AUC was the corresponding area under the plasma enantiomer concentration-time curve. Volume of distribution (V_d) was calculated by dividing corresponding CL_T by β. As the urinary excretion of AC is virtually complete ($t_{1/2} \approx 1.5$ h) within the first 24 h after single dose administration, renal clearance (CL_R) of each enantiomer was estimated by dividing the cumulative 24-hour urinary excretion of each enantiomers by the corresponding $AUC_{(0-\infty)}$ value. The overall mean concentration ratios were determined by averaging the concentrations at each time point for each rat within each group.

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of enantiomers after administration of different doses of racemate were made by a one-way ANOVA followed by Scheffe's *post hoc test*. The R:S concentration ratio was assessed for each pharmacokinetic parameter using ANOVA as above. Assumptions of homogeneity of variance were tested using the Levene test prior to ANOVA analysis. Comparisons between the S- and R-AC pharmacokinetic parameters within each study group were assessed utilizing a two-tailed student's t-test for paired data. In all tests, a probability level of significance was pre-set at $\alpha=0.05$. Results are expressed as mean ± SD.

RESULTS

Average enantiomer plasma concentration *versus* time curves after administration of varying iv doses of racemate are presented for R-AC (figure 2-1) and S-AC (figure 2-2). Pharmacokinetic parameters of R and S-AC after administration of four bolus doses of 5, 15, 30, and 50, mg kg^{-1} of racemate to rats are presented in Table 2-1.

Table 2-1. Pharmacokinetic parameters of acebutolol enantiomers after administration of racemate to rats. Data presented as mean, (SD)

Pharmacokinetic parameters	5 mg kg^{-1}		15 mg kg^{-1}		30 mg kg^{-1}		50 mg kg^{-1}	
	R-AC	S-AC	R-AC	S-AC	R-AC	S- AC	R-AC	S-AC
AUC, ug h l^{-1}	863[a] (182)	796 (136)	2085[a] (454)	1896 (354)	4754[a] (783)	4609 (879)	8847 (2021)	8846 (2163)
CL$_T$, ml min^{-1} kg^{-}	51[a] (11)	54 (10)	58[a] (8)	63 (7)	54[a] (9)	56 (11)	49 (12)	50 (13)
t$_{1/2}$, h	1.40 (0.47)	1.37 (0.41)	1.63 (0.34)	1.58 (0.34)	1.30 (0.44)	1.24 (0.38)	1.67 (0.59)	1.69 (0.72)
Cl$_R$, ml min^{-1} kg^{-1}	ND	ND	18.53[a] (3.12)	20.12 (2.90)	15.38[a] (1.60)	16.49 (1.73)	18.80 (5.76)	19.00 (6.57)
Vd, L kg^{-1}	6.10 (1.98)	6.31 (1.82)	7.33 (1.89)	7.73 (1.85)	5.95 (1.80)	5.85 (1.60)	7.50 (3.92)	7.75 (4.58)
ΣXu, mg	ND	ND	0.54 0.14	0.56 0.14	1.03 (0.22)	1.06 (0.24)	2.14 (0.40)	2.14 (0.50)
Rat weight, g	307 (14)		295 (24)		239 (10)		223 (5)	

ND, not determined;

[a] significantly different from corresponding enantiomer, $p<0.05$

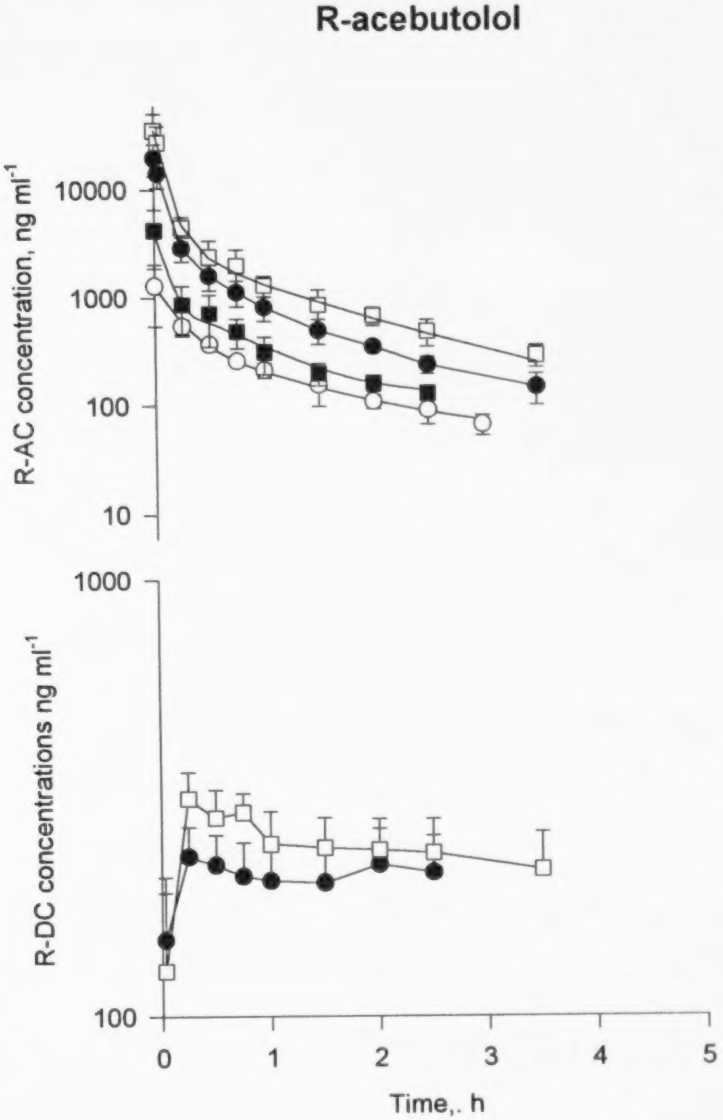

Figure 2-1. Mean plasma concentration-time profiles of R-AC and R-DC following iv administration of 5 (open circle), 15 (filled squares), 30 (filled circle), and 50 (open squares) mg kg^{-1} of racemic AC.

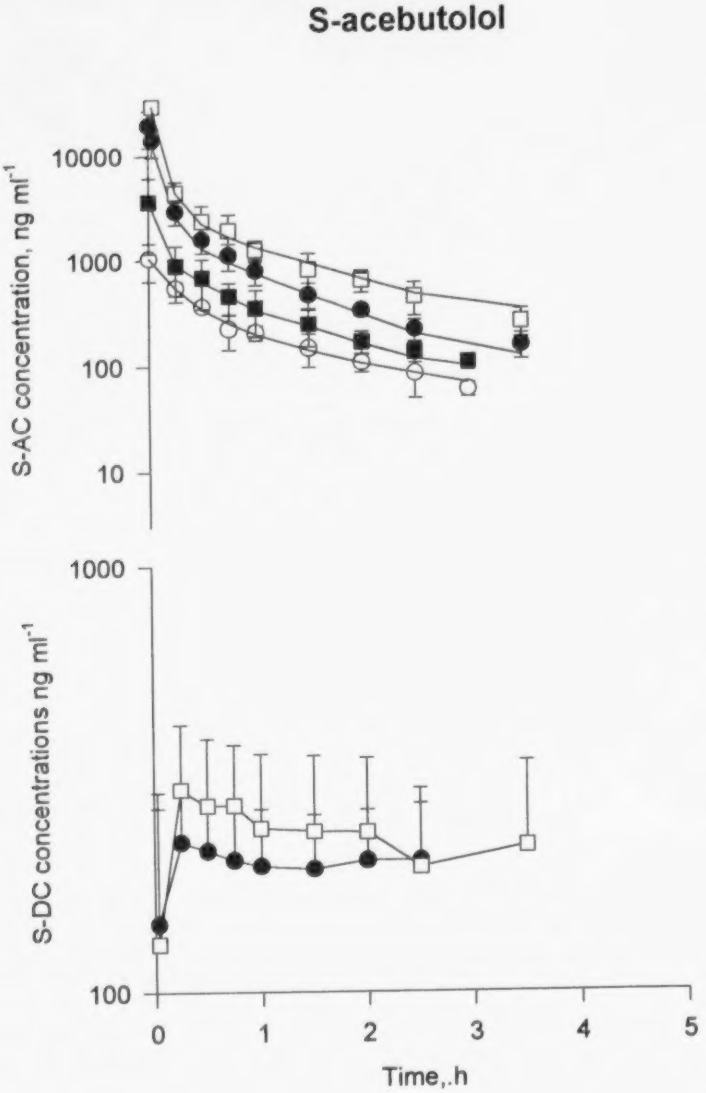

Figure 2-2. Mean plasma concentration-time profiles of S-AC and S-DC following iv administration of 5 (open circle), 15 (filled squares), 30 (filled circle), and 50 (open squares) mg kg^{-1} of racemic AC.

No significant differences between doses for CL_T, $t_{1/2}$, or V_d were observed. The disposition of AC was found to be stereoselective after administration of 5 to 30 mg kg^{-1} of racemate as the mean concentrations of R-AC were greater than that of S-AC at all measured times. Consequently, a slight but statistically significant difference in the AUC of the AC enantiomers in favor of R-enantiomer was observed. These differences were not significant after administration of 50 mg kg^{-1} of racemate.

As increasing amounts of AC were administered, the AUC increased linearly with the dose for both R-AC ($r = 0.94$, $p<0.05$) and S-AC ($r = 0.95$, $p<0.05$, figure 2-3).

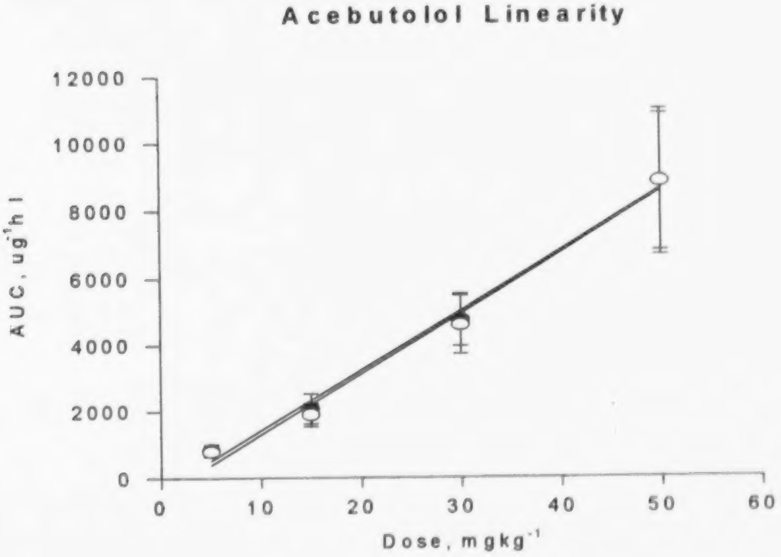

Figure 2-3. Areas under the plasma level-time curves of R-AC (filled circle) and S-AC (open circle) versus dose after administration of 5, 15, 30, and 50 mg kg-1 of racemate.

When R:S isomer ratios were evaluated, no significant differences in overall mean concentration, AUC or clearance ratios were seen among different doses. However, a trend to decreases in R:S ratios in AUC and consequently a trend to increases in this value in CL_T toward unity were observed by increasing the dose (Table 2-2).

Disposition of DC was not stereoselective as we did not find any significant differences at any time points or between the $AUC_{0\text{-}3.5}$ h of enantiomers. In fact the plasma concentrations of DC were very low, exhibiting very large variabilities and were undetectable after 5 and 15 mg kg^{-1} doses. Thus, our results are based on the plasma concentration of DC after administration of 30 and 50 mg kg^{-1} of racemate. The amount of R-DC however, was significantly greater than that of S-DC in urine after 24 h post dose (Table 2-2).

Table 2-2. Mean R:S ratios of AC and DC. Data presented as mean, (SD).

Groups	Concentration	$AUC_{0\text{-}\infty}$	$\Sigma X_{0\text{-}24}$	CL_T
A	1.06 ±0.07	1.06 ± 0.06	ND	0.92 ± 0.04
B	1.13 ± 0.10	1.06 ± 0.01	0.97 ± 0.02	0.92 ± 0.04
C	1.04 ± 0.04	1.04 ± 0.03	0.97 ± 0.02	0.97 ± 0.03
D	1.04 ± 0.06	1.00 ± 0.03	1.00 ± 0.04	1.00 ± 0.03
E	1.05 ± 0.02	1.03 ± 0.17	1.92 ± 0.4	ND
F	1.08 ± 0.15	1.07 ± 0.14	1.93 ± 0.2	ND

ND Not determined; A = AC, 5 mg kg^{-1}; B = AC, 15 mg kg^{-1}; C = AC, 30 mg kg^{-1};
D = AC, 50 mg kg^{-1}
E = DC after 30 mg kg^{-1} of AC; F = DC after 50 mg kg^{-1} of AC

The total amounts of AC recovered in urine was within the ranges of 20%-48% and 20%-51% of doses administered for R- and S- enantiomers, respectively.

Total clearance was approximately 52 and 55 ml min^{-1} kg^{-1} for R- and S- enantiomers, respectively. As we did not collect the urine for 5 mg kg^{-1} doses administrated we were unable to calculate the renal clearance for this range but the renal clearance was within the range of 14 to 29 ml min^{-1} kg^{-1} for both R- and S- enantiomers based on the 15 to 50 mg kg^{-1} doses. Furthermore, the mean fraction of drug recovered in urine as unchanged AC approximated 33 and 34 percent for R- and S-enantiomers, respectively.

DISCUSSION

The pharmacokinetics of AC and its major active metabolite DC have been investigated in man in recent years (22-25). However, there is a paucity of available information regarding the pharmacokinetic and dose range comparisons of AC enantiomers experimentally. The aim of the present study was to investigate the influence of different iv doses of AC on the pharmacokinetic profile of each enantiomer after administration of AC in the range from 5 to 50 mg kg^{-1} of racemate in rats.

Rat appears to be a good animal model to study the pharmacokinetics of AC enantiomers. The plasma concentrations of AC obtained after a 5 mg kg^{-1} and 15 mg kg^{-1} doses of AC in the rat were close to those seen in humans after 0.4 and 1.2 mg kg^{-1} (10). In agreement with our results other investigators have also found that rat is a good animal model for studying the pharmacokinetics of AC after oral administration (7,26).

Linearity study

Clinically, the disposition of many drugs follows linear kinetics and the pharmacokinetic parameters describing the disposition of drugs does not change over the therapeutic dose range. However, dose dependent pharmacokinetics have been reported for numerous drugs (27) including beta-blockers (28). The disposition of AC has also been found to be dose dependent after oral administration of AC in man (11); a 55% increase in the AUC corresponding to the last dose over that of a single dose has been reported.

The results of the present study indicate that the pharmacokinetic parameters of AC enantiomers in rats given iv AC doses between 5 to 50 mg kg^{-1} did not show any significant dose-related differences. The CL_T, V_d, $t_{1/2}$ or urinary recovery and CL_R of AC enantiomers were all linearly related to dose ($p < 0.05$). When the observed plasma concentrations (figures 2-1 and 2-2) were normalized to the doses administered, the observed concentration-time profiles following the four doses were virtually superimposable. On the other hand, a plot of the AUC *versus* dose was linearly related for AC in rat over the dose range studied for both R- ($r = 0.94$, $P<0.05$) and S- ($r = 0.95$, $P<0.05$) enantiomers (figure 2-3). These findings suggest that the disposition kinetics of AC enantiomers were linear within the dose ranges of 5 to 50 mg kg^{-1} in a rat model. Our data is in agreement with the linear disposition of

AC after intravenous administration of 1 to 10 mg kg^{-1} of AC in young healthy volunteers as reported by Meffin *et al* (10).

Stereoselective disposition and metabolism of AC in a rat model

The stereoselective disposition of drugs that have chiral centers, has been extensively documented for drugs including beta-blockers (29,30). Stereoselectivity in the clearance of beta blockers however, appears to be important for lipophilic drugs which are cleared by hepatic metabolism. Such stereoselectivity is due to differential stereochemical substrate requirements of individual hepatic cytochrome P-450 isoenzymes. Inter individual variations in the stereoselectivity can occur as a result of differences in the amount and expression of cytochrome P-450 isoenzymes due to genetic predisposition or other factors. These variations however, may depend on the species examined (38).

The results of our study as reported in table 2-1 indicated that the plasma concentrations of AC enantiomers after iv administration of racemate to rat appeared to be virtually superimposable. Slightly greater amounts of the R- as compared to the S- enantiomer of AC are seen in plasma after all doses. However, these differences were only significant following administration of 5 to 30 mg kg^{-1} iv doses. Consequently, the systemic clearance of the S-enantiomer was significantly higher than that of the antipode at these doses. Differences in the AUC values of both R- and S-AC after iv administration could be due to differences in the renal or non renal clearance pathways. Following iv administration of AC, we found that the renal clearance of AC exceeded 5 ml min^{-1} kg^{-1}, the reported glomerular filtration rate (31) for both enantiomers, which is suggestive of renal tubular secretion of AC in rat. As active tubular secretion could contribute to stereoselectivity in drug disposition (32,33), the differences in the AUC values and, consequently, in the total clearance of AC enantiomers could be explained by differences in renal clearance of enantiomers. Our data for the 15 to 50 mg kg^{-1} doses indicated that CL_R values for S-AC tended to be greater than that of R-AC (Table 2-1). This difference, however, was only significant following administration of the 15 and 30 mg kg^{-1} doses. Stereoselective disposition due to renal clearance has also been reported for atenolol (32,33).

Although non-renal clearances of AC including hepatic metabolism (34,35) could also be responsible for the observed stereoselectivity, differences between clearance values of R- and S-AC were not significant.

The effect of different doses on enantiomeric disposition of AC were also evaluated in the present study for individual data points, AUC and CL_T. Although a trend to decreases in R:S ratios toward unity for AUC were observed by increasing the dose, the concentration, AUC or CL_T enantiomers ratios show no significant differences among different doses (Table 2-2). A change in enantiomeric ratio by increasing the dose has been reported for other drugs including MK-927, a carbonic anhydrase inhibitor (36). A decrease in the clearance of the R-enantiomer from 40 times that of S-enantiomer at 0.05 mg kg^{-1} to 7-fold at 5 mg kg^{-1} has been reported following administration of MK-927.

The plasma concentrations of DC were very low and undetectable after 5 and 15 mg kg^{-1} doses. The metabolite was measured after 30 and 50 mg kg^{-1} with no significant statistically (Table 2-2). The amounts of R-DC collected in urine were, however, significantly greater than that of S-DC (Table 2-2). This finding could be explained by stereoselective renal excretion of R-DC and/or the higher formation of R-DC *via* the kidney, as it has been reported that DC is cleared completely through the kidneys (37). The same results have been reported for the kinetics of DC in human (6).

The total amount of unchanged AC recovered (0-24 h) in urine was about 33% and 34% of the administered dose for R-and S-enantiomers respectively. These data are consistent with a previous report on human after iv administration of drug which reported approximately 40% for this value (10).

In conclusion, this study undertook to investigate the pharmacokinetics of AC and its major metabolite DC over a dosage range which yielded plasma concentrations similar to humans. Our results indicated that rat may be a good animal model for pharmacokinetic studying of AC as the plasma concentrations of AC obtained after iv administration of 5 mg kg^{-1} and 15 mg kg^{-1} doses were in close range to those seen in humans after iv doses of 0.4 and 1.2 mg kg^{-1} (10). Further, AC follows linear pharmacokinetics in rat after iv administration of racemic doses of 5 to 50 mg kg^{-1}. We found that the disposition of AC was stereoselective, however, the data suggest that there is a tendency for stereoselectivity to be lost at higher doses. DC plasma concentrations were low with respect to AC, the data suggest a stereoselective renal clearance for DC. Non renal clearance however, was not stereoselective. Further, AC suited for use in patients in whom iv beta blockade is desirable as the basic pharmacokinetic parameters obtained after the iv bolus doses can be used to design iv infusion regimens.

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✱CHAPTER 3✱

Pharmacokinetics of Single Oral and Multiple Intravenous and Oral Administration of Acebutolol Enantiomers in a Rat Model[♦]

INTRODUCTION

Acebutolol (AC), a chiral β-adrenergic blocking agent has been extensively used worldwide for many years. It is a cardioselective β-blocker which also possesses intrinsic sympathomimetic activity (ISA, 1).

Although AC is used clinically as the racemate, data describing the stereospecific pharmacokinetics of AC after multiple doses are sparse. In a study by Gulaid *et al.* (2) in man, it was reported that the mean area under the plasma concentration-time curve (AUC) of AC for a dosing interval during repeated oral (P.O.) dosing was about 70% greater than the corresponding $AUC_{0-\infty}$ for the single P.O. dose. In another study a 55% increase in this parameter was reported (3). However, these studies analyzed AC utilizing non-stereospecific methods and were therefore unable to reveal the disposition of individual enantiomers. Thus, the occurrence of any enantiomeric change in disposition of AC after multiple dosing remains unknown. As previously pointed out by others (4-6), conclusions regarding

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disposition of chiral compounds can only be made after examining enantiomeric disposition.

Our laboratory has recently reported the stereospecific pharmacokinetics of AC enantiomers in rats, and found that the pharmacokinetics of AC after iv administration of racemate is stereoselective and linear over the dose range studied in this species (7). Furthermore, the disposition of AC is stereoselective after single oral administration of racemate in man and rat (8,9). However, the pharmacokinetics of enantiomers may be different after chronic administration of the drug as the influence of any putative physiologic changes of the disposition of AC is not well defined. Thus, we sought to determine the enantiomeric disposition of AC after single and multiple administration of racemate. As it has been reported that rat may be a good animal model for studying the pharmacokinetics of AC we used this species (7,9,10).

MATERIALS AND METHODS

Chemicals

Racemic AC and the internal standard (IS), racemic pindolol, was bought from Sigma (Illinois, USA). Racemic diacetolol (DC) was obtained from Rhone-Poulenc, Rorer Canada Inc. (Montreal, Que, Canada). All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance

Male Sprague-Dawley rats (n= 6 in each group) were kept on a light-dark cycle of 12 hours at a room temperature of 25° C. They were fasted for about 8 hours before and two-hours following drug administration for the single dose study and also before the last dose of multiple dosing, with free access to water. Animals were catheterized with silastic tubing (0.025 in .i.d. × 0.037 in. o.d.; Dow Corning, Midland, MI, USA) at the right jugular vein while they were under general anesthesia with pentobarbital administered *via* the intraperitoneal route. They were individually housed in 18 in.× 9.5 in.× 8 in. polycarbonate rodent cages and allowed to recover overnight prior to the experiments.

Dosing and sample collection

Racemic AC, dissolved in normal saline (one ml kg^{-1}), was administered either as iv dose of 30 mg kg^{-1} or as P.O. dose of 50 mg kg^{-1} *via* the jugular vein or by gavage every eight hour for four days. The same solution was used for a single P.O. dose of 50 mg kg^{-1} of racemate. Blood (0.25 ml) was collected from the jugular vein cannula just prior to and at 2, 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3.5, and 5.5 h after drug administration. Between each blood sample collection 0.25 ml normal (0.9%) saline was administered *via* the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at -20°C until analyzed.

Urine was also collected and pooled for 24 h following every treatment. Urine was kept frozen at -20°C until just prior to analysis.

Stereospecific HPLC assay of AC and DC

Concentrations of R- and S-AC and R- and S-DC in plasma and urine were determined utilizing a previously reported stereospecific HPLC method (11,12). For urine sample analysis, specimens were diluted 1:10 (v/v) in HPLC grade water prior to extraction, derivatization and subsequent analysis.

Pharmacokinetic data analysis.

The AUC was calculated by the linear trapezoidal rule. The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points in the terminal elimination phase. This procedure could not be followed in the case of metabolite due to its low concentration in plasma. Thus, AUC values reported for DC are the AUC from zero to 3.5 h ($AUC_{0\text{-}3.5}$). The terminal elimination half-life ($t_{1/2}$) of AC was calculated by $0.693/\beta$. Total body clearance (CL_T) was calculated as $D/AUC_{0\text{-}\infty}$ after iv ($AUC_{i.v.}$) administration, where D was the enantiomeric dose administered. Oral clearance (CL/F) was calculated by dividing the total administered enantiomeric dose with the AUC after P.O. administration ($AUC_{p.o.}$). The fraction of the dose reaching the systemic circulation (F) was estimated by dividing the $AUC_{p.o.}$ by the $AUC_{i.v.}$ after administration of the same dose. Maximum plasma concentrations (C_{max}) and the time required to reach the maximal concentrations (T_{max}) were obtained directly from graphical analysis of plasma concentrations *vs.* time. Volume of distribution (Vd_{β}) was calculated by dividing corresponding CL_T by β. Renal clearance (CL_R) of each enantiomer was estimated by dividing the cumulative 24-hour urinary excretion of them by the corresponding $AUC_{0-\infty}$ value.

After multiple iv administration of AC the CL_T was calculated as $D/AUC_{0-\tau}$ where $AUC_{0-\tau}$ was the corresponding AUC within a dosing interval at plateau. CL/F was calculated by dividing the total administered enantiomeric dose with the $AUC_{0-\tau}$ after multiple P.O. administration. CL_R of each enantiomer was estimated by dividing the amount of each enantiomer excreted unchanged in urine in a dosing interval at steady state by the value of $AUC_{0-\tau}$.

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of AC enantiomers either after single or after multiple dose administration of AC were assessed utilizing a two-tailed student's t-test for paired data. Statistical comparison of pharmacokinetic data of AC after single iv *vs.* single P.O. and single *vs.* multiple dose studies for R- *vs.* R- and S- *vs.* S-AC were made by a two tailed student's t-test for two samples assuming equal variance. In all tests, a probability level of significance pre-set at $\alpha = 0.05$. Results are expressed as mean ± SD.

RESULTS

Single oral dose

Mean plasma concentrations of R- and S-AC and R- and S-DC after a single P.O. dose are shown in Fig. 3-1. The pharmacokinetic parameters of R- and S-AC are summarized in Table 3-1. The time-courses of the two AC enantiomers were stereoselective after P.O. administration of AC to rats. The C_{max} was attained at 1.63 ± 0.62 h for both enantiomers and declined with a half-life of approximately 1.9 and 1.8 h for R- and S-enantiomer, respectively. Both enantiomers were rapidly absorbed with bioavailability values of approximately 0.36 and 0.34 for R- and S-enantiomers, respectively. The concentration *vs.* time profile was characterized by the appearance of two peaks for both enantiomers, at about 15 min. and between 1-1.5 h after P.O. administration.

Very low concentrations of DC were found in plasma in this species after single P.O. administration of AC, however, the disposition of DC was stereoselective in favor of R-enantiomer. The $AUC_{0\text{-}3.5}$ for R-enantiomer was significantly greater than that of its antipode. The amount of R-DC collected in urine was significantly greater than that of its antipode (Table 3-2).

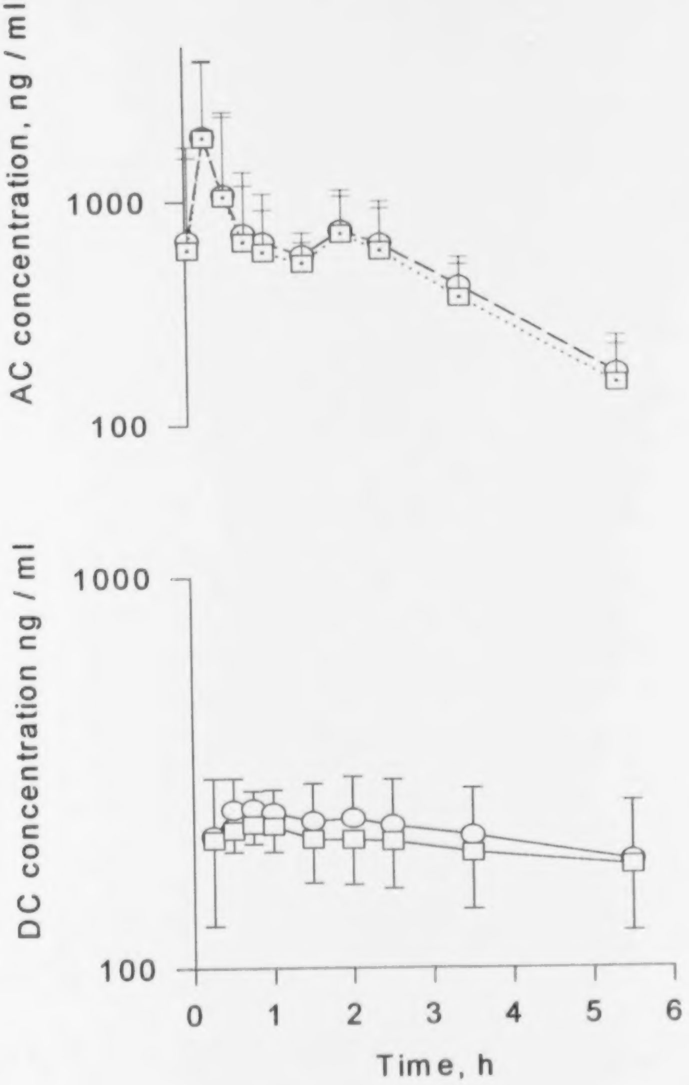

Figure 3-1. Mean plasma concentration-time profiles of R and S-AC and R- and S-DC following P.O. administration of 50 mg kg^{-1} of AC to rat. Key: R-enantiomer = open circles; S-enantiomer = open squares.

Table 3-1. Pharmacokinetic parameters of acebutolol enantiomers after administration of racemate to rats. Data presented as mean, (SD).

Pharmacokinetic parameters	Intravenous Administration, 30 mg kg^{-1}				Oral Administration 50 mg kg^{-1}			
	Single dose		Multiple dose		Single dose		Multiple dose	
	R	S	R	S	R	S	R	S
^{a}AUC, µg h l^{-1}	4754^{*} (783)	4609 (879)	5160 (1285)	4972 (1380)	$3155^{*\#}$ (1003)	$3007^{\#}$ (895)	4409 (514)	4390 (530)
$^{b}CL_T$, ml min^{-1} kg^{-1}	54^{*} (9)	56 (11)	51 (13)	54 (15)	$138^{*\#}$ (39)	$149^{\#}$ (42)	95 (10)	96 (13)
$t_{1/2}$, h	1.30 (0.44)	1.24 (0.38)	1.12 (0.34)	1.14 (0.36)	1.92 (0.60)	1.86 (0.60)	2.04 (1.18)	1.99 (1.14)
CL_R, ml min^{-1} kg^{-1}	15.38^{*} (1.60)	16.49^{*} (1.73)	15.09 (3.70)	15.81 (4.14)	24.63 (5.56)	25.25 (6.43)	21.96 (4.64)	21.57 (5.19)
$^{c}V_d$, L kg^{-1}	5.95 (1.80)	5.85 (1.60)	4.79 (1.51)	5.08 (1.58)	22.70 (8.59)	23.61 (8.55)	17.48 (11.32)	17.22 (11.72)
ΣX_U, mg	1.03 (0.22)	1.06 (0.24)	1.13 (0.17)	1.12 (0.17)	1.13 (0.65)	1.08 (0.64)	1.90 (0.42)	1.86 (0.41)
Rat weight, g	239 (11)		257 (8)		223 (17)		337 (34)	

*significantly different from corresponding enantiomer, $p<0.05$

$^{\#}$ significantly different from corresponding enantiomer after multiple dose, $p<0.05$; a. $AUC_{0-\tau}$ after multiple dosing; b. CL / F after oral administration; c. V_d / F after oral administration; ΣX_U = Comulative urinary excretion

Table 3-2. Pharmacokinetic parameters of diacetolol enantiomers after administration of acebutolol to rats. Data presented as mean, (SD).

Pharmacokinetic parameters	Intravenous Administration, 30 mg kg^{-1}				Oral Administration, 50 mg kg^{-1}			
	Single dose		Multiple dose		Single dose		Multiple dose	
	R	S	R	S	R	S	R	S
$AUC_{0-3.5}$, µg h l^{-1}	699 (304)	656 (200)	1463 (265)	1441 (355)	$791^{*\#}$ (179)	$719^{\#}$ (149)	1539^{*} (361)	1382 (307)
$t_{1/2}$, h	ND	ND	ND	ND	ND	ND	4.07 (1.28)	4.21 (1.05)
$(\Sigma X_U DC/\Sigma X_U AC)\%$	6.40^{*} (1.06)	3.27 (0.49)	8.31^{*} (1.46)	3.51 (0.25)	$17.49^{\#*}$ (7.11)	$9.23^{\#}$ (4.34)	7.0^{*} (1.70)	3.08 (0.55)
ΣX_U, µg	66^{*} (19)	34 (4)	89^{*} (19)	37 (8)	165^{*} (49)	80 (27)	132^{*} (39)	58 (16)

ND Not identified due to low concentration of DC.

*significantly different from corresponding enantiomer, $p<0.05$.

$^{\#}$ significantly different from corresponding enantiomer after multiple dose, $p<0.05$.

ΣX_U = Comulative urinary excretion

Multiple dosing regimen

The pharmacokinetic parameters of AC enantiomers after multiple iv and P.O. administration are summarized in Table 3-1. There were no significant differences between $AUC_{0-\infty}$ of AC enantiomers after single iv or $AUC_{0-\tau}$ after multiple iv administration of racemate and the time courses of AC enantiomers in plasma were virtually superimposable (Fig. 3-2). However, this value was increased significantly after multiple P.O. administration of AC compared with the corresponding $AUC_{0-\infty}$ after single P.O. dosing (Fig. 3-3). Oral clearance after multiple P.O. administration of racemate was, 96 ± 10 and 96 ± 13 ml min^{-1} kg^{-1} for R- and S-AC, respectively. This value was significantly different from that of the corresponding enantiomers after single P.O. administration of AC (Table 3-1). The $t_{1/2}$ of both enantiomers at steady state were similar to those after single-dose administration of AC either after iv or after P.O. dosing.

Two concentration maxima were absent after multiple iv administration. It was observed however, after multiple P.O. administration of the drug. Thus, the plasma concentrations of AC enantiomers reaches a maximum in the first hour and 2 h after the administration of last P.O. dose.

After multiple administration of AC the $AUC_{0-3.5}$ for DC was significantly higher than that after single administration of AC either after iv or after P.O. dosing for both enantiomers. Furthermore the amount of R-DC collected in the urine was significantly greater than that of S-DC (Table 3-2).

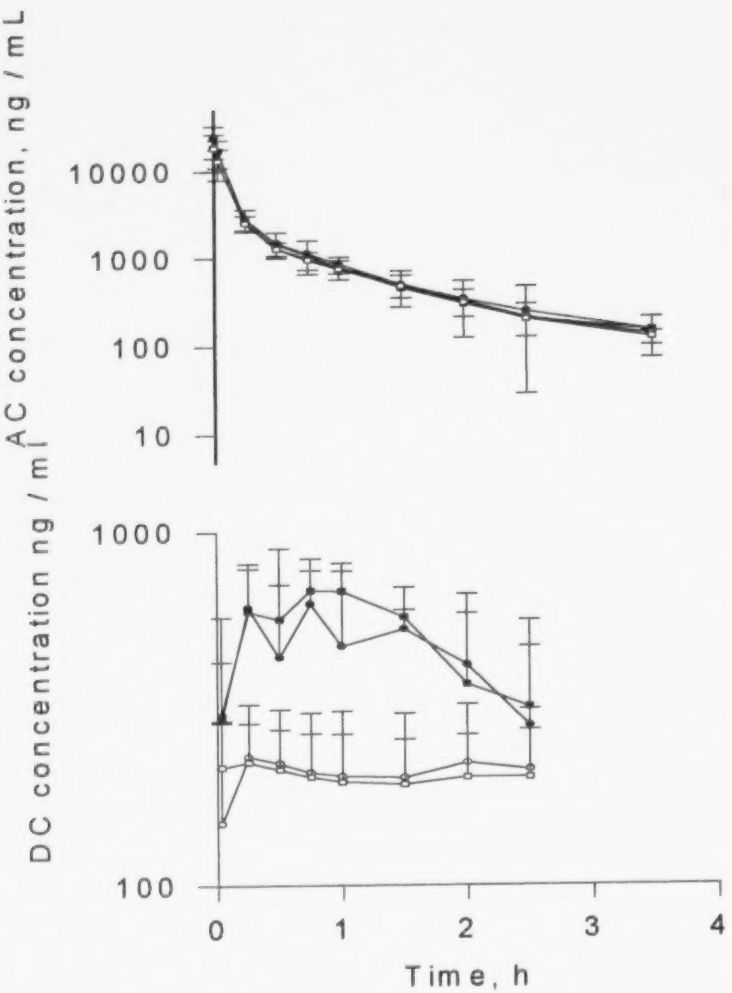

Figure 3-2. Mean plasma concentration-time profiles of AC and DC enantiomers following iv single and after the last dose of multiple administration (thrice daily for four days) of 30 mg kg^{-1} of racemate to rat. Key: R-enantiomer after single dose = open circles; R-enantiomer after multiple dose = filled circles; S-enantiomer after single dose = open squares; S-enantiomer after multiple dose = filled squares.

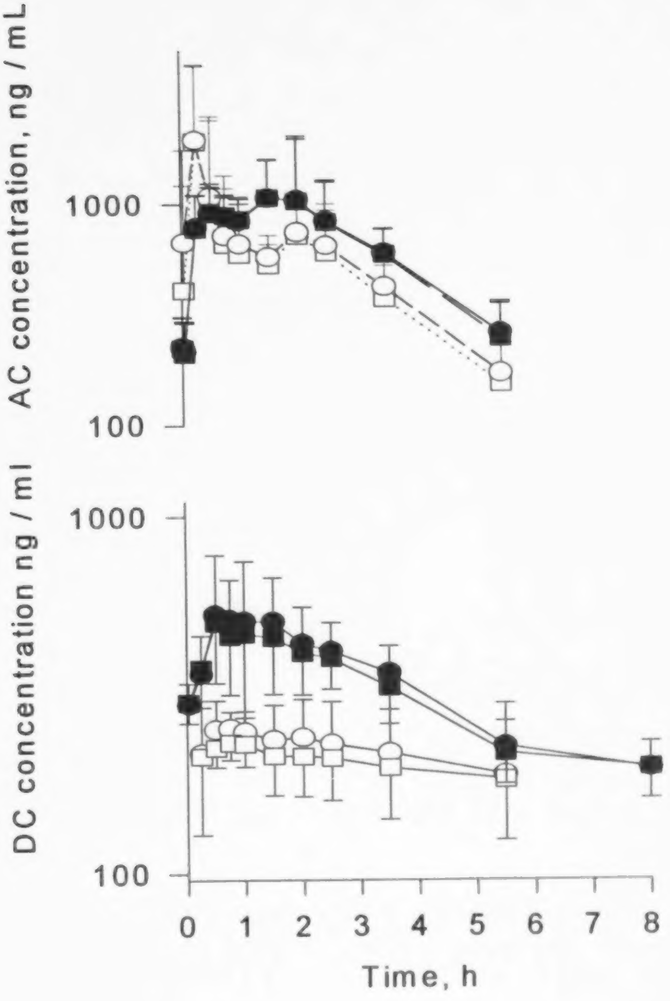

Figure 3-3. Mean plasma concentration-time profiles of AC and DC enantiomers following oral single and after the last dose of multiple administration of 50 mg kg^{-1} of racemate to rats. Key: R enantiomer after single dose = open circles; R-enantiomer after multiple dose = filled circles; S-enantiomer after single dose = open squares; S-enantiomer after multiple dose = filled squares.

Due to low concentrations of DC for the terminal elimination phase, we were unable to calculate the $t_{1/2}$ of DC after iv or single P.O. administration of AC. This value however, was 4.1 ± 1.3 and 4.2 ± 1.1 after multiple P.O. administration of AC (Table 3-2).

DISCUSSION

When there is a lack of superposition on administering a drug on separate occasions or a lack of predictability following repeated or continuous dosing, based on single dose data, the drug is said to depict time-dependent kinetics. Such behavior could be a source of variability in drug response. There are numerous examples in the literature describing the time-dependent pharmacokinetics of drugs (13,14). The most common cause of time-dependent kinetics are variations in enzyme induction, diurnal variations in renal function, urine pH, α_1-acid glycoprotein concentration, gastrointestinal physiology, cardiac output and regional blood flow (15).

We previously reported the enantiomeric disposition of AC following iv administration of racemate to rats (7). In that study, it was concluded that the disposition of AC was not dose-dependent after iv administration of AC over a given dose range. To test whether the pharmacokinetic parameters of AC may be time-dependent, the enantiomeric disposition of AC was investigated after single and multiple dose administration of iv and P.O. doses of AC to rats.

Single oral dose

After single P.O. administration of the racemate, the pharmacokinetics of R- and S-AC in rats were stereoselective. Slightly but significantly greater amounts of the R-enantiomer of AC were seen in plasma after a 50 mg kg^{-1} P.O. dose (AUC R:S 1.08 ± 0.04). AC undergoes first-pass metabolism to DC, such that only 35% of a dose is systemically available in man (16). This pathway may be saturable as the pharmacokinetics of AC is reported to be dose-dependent after P.O. (3), but not after iv administration (7,17). Furthermore, based on previously reported values for $AUC_{0-\infty}$ for AC enantiomers (7) and the results of this study, absolute bioavailabilities of 0.36 and 0.34 were calculated for R- and S-AC, respectively. Consequently, the extraction ratios of 0.64 and 0.66 were calculated for AC enantiomers indicating that AC undergoes a moderate degree of first pass metabolism assuming complete absorption through the gastrointestinal tract (18). The 36% and 34% P.O. bioavailabilities estimated for R- and S-AC from our data are consistent with those reported in man (16).

With regard to the metabolite, we found that the concentrations of DC enantiomers in plasma were very low in rat. This is contrary to what is observed in humans (1,2). Hence, considering equal bioavailability in rats with humans (16) other metabolites may be involved in AC disposition in rat. It has been reported that AC is primarily metabolized via hydrolysis of the butyramide group to form acetolol, followed by N-acetylation to DC. After P.O. administration of AC in man the intermediate metabolite, acetolol, accounts for less than 10% and DC for more than

60% of recovered drug in urine. However, acetolol has been collected as a major metabolite (30%) in rat urine (19). Nevertheless, larger amounts of R-DC as compared to its antipode were found (Table 2) in rat urine. This suggests that either the biotransformation of AC to DC is greater for the R-enantiomer or the renal clearance of DC is stereoselective in favor of R-enantiomer in rat model.

It was found that the $AUC_{0-3.5}$ of DC in plasma and consequently the amount of metabolite which was recovered in the urine were not significantly different after P.O. from those after iv administration of racemate in rat (Fig 3-3). These observations may suggest that the fraction of the dose converted to DC is independent of route of administration in the rat and also it implies that the drug is efficiently absorbed from the rat gastrointestinal tract which is similar to that of human (18). Given that ingested drug entirely traverses the gastrointestinal wall and considering the equality of AUCs of DC following P.O. and iv administration of AC, it may be concluded that this metabolite, primarily if not totally, is formed in the liver of rat. Similar results have been described by other investigators in man (16).

The interesting point observed after P.O. administration of AC was the existence of multiple peaks in the absorptive phase of our rats. Multiple peaking is often attributed to enterohepatic recycling (20), discontinuous gastrointestinal absorption (21-23), formation of poorly absorbed micelle complex of drug with bile salts (24,25), variable gastric emptying (26,27), gastric pH (28), or reversible metabolism (29). These theories are reported for other drugs however, they are yet to be proven for AC. As the multiple peaks phenomenon was not observed after iv administration of AC (Fig. 2, 4) it is unlikely that enterohepatic recirculation plays an important role in the appearance of double peaks after P.O. dosing as it has been reported by others (10). Thus, the aforementioned possibilities remains to be proven as a possible mechanisms for this phenomenon.

Multiple dosing

The principle of superposition assumes that $AUC_{0-\infty}$ following a single dose of drugs with linear pharmacokinetics is equal to the $AUC_{0-\tau}$ at steady state following administration of the same dose. Gulaid *et al* (2) who compared the plasma concentration time profiles from single (400 mg P.O.) and multiple (400 mg, b.i.d P.O.) doses of AC in man have shown that the mean AUC of AC for a dosing interval during repeated dosing was about 70% greater than the corresponding $AUC_{0-\infty}$ after single dose administration. Similar results have also reported by others (3). Although no significant differences for $AUC_{0-\tau}$ were observed after multiple iv administration of AC as it compared with single dose data, the 40% and 46% increases in mean $AUC_{0-\tau}$ during multiple P.O. dosing compared with the mean $AUC_{0-\infty}$ for the single P.O. dose of AC was observed for both R- and S-AC, respectively. Thus, we concluded that the superposition principle for AC enantiomers after single and multiple dose administration of racemate has been established only after iv administration of this drug.

There are several possible explanations for these observations. The AUC is determined by bioavailability and clearance, therefore, alteration in one of these parameters at steady state would result in differences between $AUC_{0-\infty}$ after single dose and $AUC_{0-\tau}$ at steady state.

One explanation revolves around the clearance of AC, which depends on both renal and non renal processes. The renal clearance of AC exceeded (5 ml min^{-1} kg^{-1}), the reported glomerular filtration rate (30) after single iv dosing, for both enantiomers. This suggests that the renal tubular secretion of AC may occur in rat. As the renal secretion of drugs is an active process it is possible that AC undergoes concentration-dependent renal secretion resulting in lower renal clearance at steady state. Nevertheless, we did not find any significant differences in renal clearance of AC either after multiple iv or after multiple P.O. administration of AC as compared with single dose. Therefore, it seems unlikely that changes in renal secretion would result in the differences in $AUC_{0-\infty}$ and $AUC_{0-\tau}$ shown in this study. The other possibility for the change in clearance of AC is a change in non renal clearance. As AC is moderately extracted by the liver, any changes in either enzyme activities or blood flow through the organ could contribute to this alteration in clearance. To our knowledge, there is no report on any enzymatic changes in liver function induced by AC. However, the fall in blood pressure during treatment with some β-blockers is associated with a change in cardiac output (31). Nonetheless, the fall in blood pressure during treatment with β-adrenoceptor blockers with ISA such as pindolol or AC is associated with a decrease in vascular resistance, but cardiac output either does not change or the change is minimal (32). In fact, changes in cardiac output may alter blood flow through the eliminating organs such as liver or kidney resulting in changes in the clearance of drugs which are eliminated by these organs. With AC the changes in enzymatic activities or blood flow after multiple dosing is unlikely as we did not find any significant differences either in $AUC_{0-\infty}$ or CL_{nr} after single iv administration as compared with the $AUC_{0-\tau}$ after multiple iv dosing.

A second possible explanation for our findings involves bioavailability, which was about 34% and 36% after single dose for R- and S-AC, respectively. However, it was increased to 51% and 53% after multiple administration of racemate for R- and S-AC, respectively. Furthermore, P.O. bioavailability is a function of pre-systemic extraction by enzymatic metabolism primarily on first liver passage. The bioavailability of AC is low because of this first pass as it has been reported that after P.O. administration, AC is almost completely absorbed (1). Therefore, saturation of the first-pass metabolism appears the most likely explanation for the increase in bioavailability of AC observed after multiple dosing.

Interestingly, we found that the ratio of DC to parent drug recovered in the urine was lower after multiple P.O. dosing compared with the single dose data (Table 3-2). This may suggest that the CL_{int} is reduced after multiple dosing. A decrease in intrinsic hepatic clearance would be expected to increase bioavailability at steady state with little or no change in systemic clearance. This is consistent with the lack of change in β at steady state compared with that after single dose (Table 3-1). Another possible explanation for a change in bioavailability after multiple dosing is a change in blood flow due to this multiple dosing. Tam (33) has shown that the venous equilibration model predicts no effect of blood flow on concentration of drug at steady state after multiple P.O. dosing (C_{po}^{ss}) whereas, the undistributed sinusoidal model predicts that C_{po}^{ss} is sensitive to blood flow change. However, the sensitivity depends on the extraction ratio. Together with the facts that AC is a moderately extracted drug and possesses some degree of ISA make it to be less sensitive to perturbations in physiological parameters such as cardiac output or blood flow. Thus the effect of blood flow on C_{po}^{ss} for AC even if we predict it with the undistributed sinusoidal model is either negligible or improbable. However, this needs more investigation to be proved after blood flow measurements in rat.

As with the single P.O. dose, following multiple dose administration of AC we observed the multiple peaking phenomenon.

In conclusion, our results indicate that AC is a moderately extracted drug in rat. Furthermore, the pharmacokinetics of AC at steady state could be predicted by using the single dose data only after iv administration as after multiple P.O. administration of racemate it accumulates.

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# *CHAPTER 4*

Influence of Cimetidine Administration on the Pharmacokinetics of Acebutolol and Diacetolol Enantiomers

INTRODUCTION

Acebutolol (AC) is a chiral β-adrenergic receptor antagonist which is widely used in the treatment of hypertension (1) and cardiac arrhythmias (2). Although AC is manufactured as the racemate, its β-blockade activity resides predominantly with S-enantiomer (3). AC is rapidly absorbed in rat (4) and human (5, 6) with similar extents of absorption for both enantiomers (7). Peak plasma concentrations and the area under the plasma concentration time curves (AUC) of AC enantiomers are different among individuals suggesting stereoselective first-pass metabolism for AC in man (7,8) and rat (4). When AC is administered orally, the plasma concentration time profile frequently shows two maxima (9), the first at approximately 0.25 h and the second at approximately 1.5 h in rat (4). Although, there is no report on multiple peak phenomena in human for AC, the "erratic" plasma concentration-time profile of AC (5, 10-14) and DC (15) has been reported after P.O. administration. As expected, a double-peak effect disappears if the drug is administered intravenously (4, 16-18). Double peaks have been observed in the plasma concentration profile of several other drugs including acetaminophen (19), aspirin (20), cimetidine (21), furosemide (22), penicillamine (23), and ranitidine (24), following P.O. administration.

Several mechanisms have been suggested for occurrence of more than a single concentration maxima including the effect of gastric emptying (19). Gastric emptying is altered due to drugs and disease (25). Drugs may alter the rate of gastric emptying by effects on the smooth muscle, influencing the release of intestinal hormones which modulate gastric activity or by influencing the gastric pH. It has been reported that gastric emptying is reduced (26) by both weak (*e.g.* lactic, tartaric and ascorbic acids) and strong acids (*e.g.* hydrochloric and nitric acids) while the basic (*e.g.* sodium bicarbonate or disodium phosphate) solutions were found to increase the rate of emptying in healthy subjects (27). Therefore, elevation of gastric pH may increase the gastric emptying rate, with a substantial amount of the drug emptying into the duodenum soon after administration. The fast gastric emptying may produce a rapid increase in plasma concentration and also result in the appearance of a single peak in the plasma profile of drugs. The disappearance of multiple peaks due to increasing pH has recently been reported for CIM (28).

The objective of this study was to determine whether a change in gastric emptying due to a change in gastric pH plays an important role in the generation of double peaks following P.O. administration of AC. CIM was administered in order to increase intragastric pH. We further addressed the influence of CIM co-administration on the pharmacokinetics of AC and DC enantiomers in the rat model. This study was conducted in rat as it has been shown to be a good animal model for studying the double peak phenomena of AC (9).

MATERIALS AND METHODS

Chemicals

CIM, racemic AC and the internal standard (IS), racemic pindolol, were bought from Sigma (Illinois, USA). The metabolite, (DC, as hydrochloride salt) was obtained from Rhone-Poulenc Rorer Canada Inc. (Montreal, Que, Canada). All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance.

Male Sprague-Dawley rats weighing between 220-350 g were chosen for this study. Rats were fasted for about 8 hours prior to, and for two hours following the AC administration, with free access to water. Animals were anesthetized with pentobarbital (ip) and right jugular vein was catheterized with silastic tubing (i.d. = 0.025, o.d. = 0.037 Dow Corning, Midland, MI, USA). The animals were allowed to recover overnight prior to the experiments. During this time the animals were individually stored in 18 in. × 9.5 in. × 8 in. polycarbonate rodent cages.

Dosing and sample collection

CIM was administered as a bolus 50 mg kg^{-1} dose in normal saline at time zero to raise intragastric pH. After 30 minutes, racemic AC dissolved in normal saline was administered orally in a dose of 50 mg kg^{-1}. Blood (0.25 ml) was collected from the jugular vein cannula at 0, 2, 15, 30, and 45 min. and at 1, 1.5, 2, 2.5, 3.5, 5.5 and 8 h after AC administration. Between each blood sample collection 0.25 ml normal saline was administered via the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at -20°C until analyzed. Urine was collected and pooled for 24 h following drug administration. Urine samples were kept frozen at -20°C until analyzed.

Stereospecific HPLC assay of AC and DC

Concentrations of R- and S-AC and R- and S-DC in plasma and urine were determined utilizing a previously reported stereospecific HPLC method (29,30,31). For urine sample analysis, specimens were diluted 1:10 (v/v) in HPLC grade water, derivatized, and subsequently analyzed.

Pharmacokinetic data analysis

The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule. The area from the last concentration point C_{last} to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points in the terminal elimination phase. We could not calculate the β for metabolite due to low concentration. Therefore, the AUC values reported for DC are the AUC from zero to 3.5 hour post dose. The terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\beta$. The oral clearance ($Cl_{p.o.}$) was calculated as $D/AUC_{0-\infty}$, where D was the enantiomeric dose administered. The volume of distribution (Vd / F) was calculated by dividing corresponding $Cl_{p.o.}$ by β. As the urinary excretion of AC is almost complete within the first 24 h after single dose administration, renal clearance (Cl_R) of each enantiomer was estimated by dividing the cumulative 24-hour urinary excretion of each enantiomers by the corresponding $AUC_{0-\infty}$. The relative bioavailability was calculated by dividing enantiomeric $AUC_{0-\infty}$ of AC after co-administration of CIM over the same value after administration of AC alone

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of enantiomers after administration of racemate were made by a two-tailed student's t-test for paired. Statistical comparison of pharmacokinetic data of AC after co-administration of CIM *vs.* administration of AC alone for R- *vs.* R- and S- *vs.* S-AC were made by a two tailed student's t-test for two samples assuming equal variance. Significance was assumed at 5% level. Results are expressed as mean ± SD.

RESULTS

The plasma concentration *vs.* time profiles of AC exhibited two concentration maxima after P.O. administration. CIM co-administration had no effect on the appearance of this phenomenon for either R- or S-AC (Figs. 4-1,4-2).

Pharmacokinetic parameters of R- and S-AC after co-administration of CIM (50 mg kg^{-1}) to rat are summarized in Table 4-1. A slight but statistically significant difference in the AUC of the AC enantiomers in favor of R-AC was observed after administration of CIM. Co administration of CIM resulted in lower t_{max} value for AC enantiomers. The $CL_{p.o.}$ of AC enantiomers did not change significantly in co-administration with CIM although a trend towards increase in this value was observed.

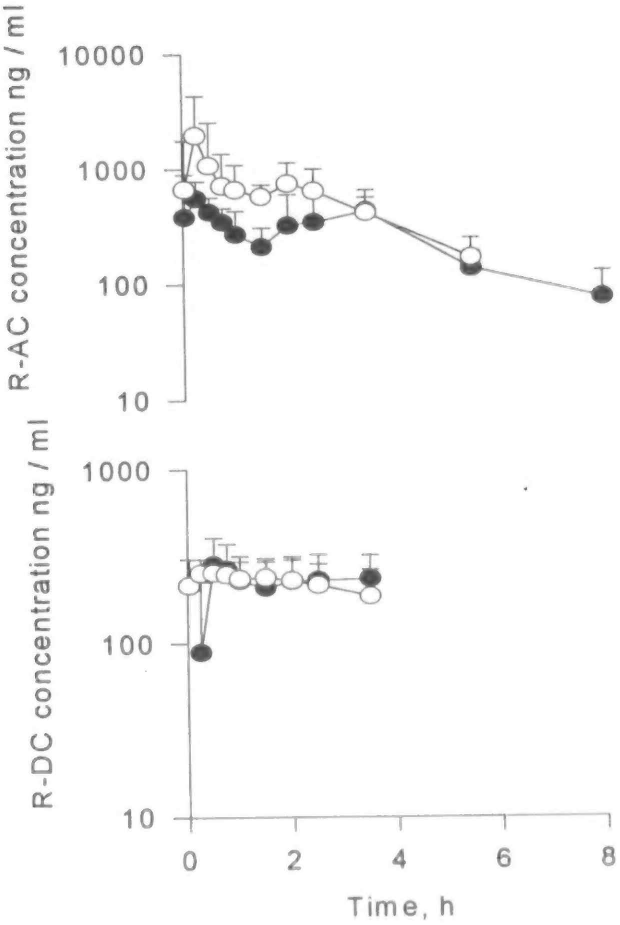

Figure 4-1. The average plasma concentration versus time profiles for R-AC and R-DC after administration of AC alone (open circle) and after co-administration of cimetidine (filled circle).

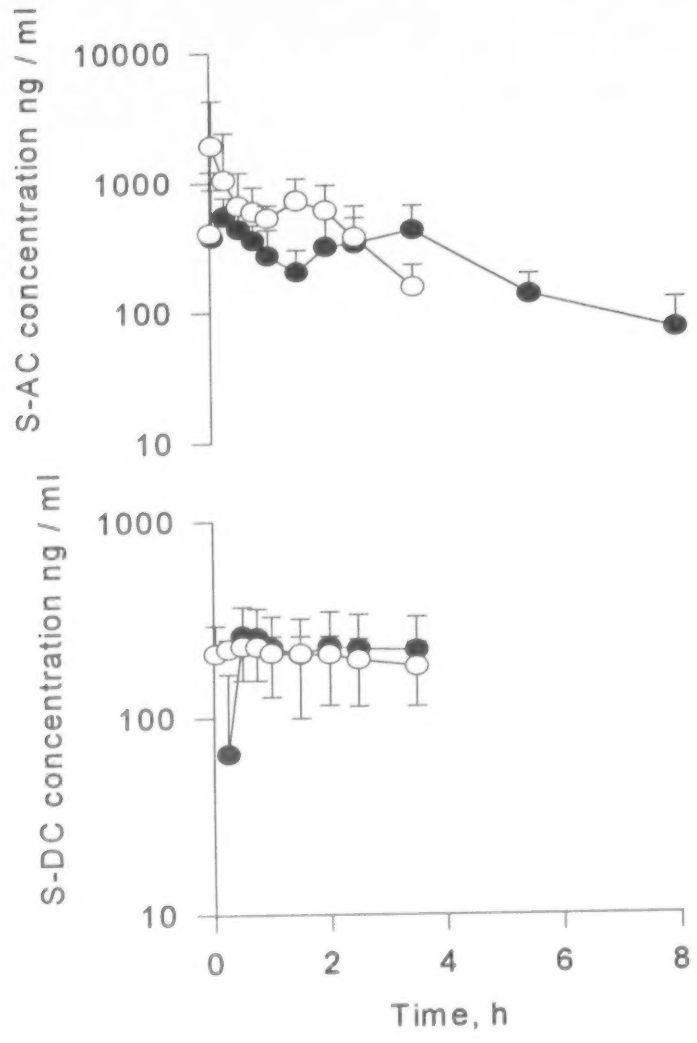

Figure 4-2. The average plasma concentration versus time profiles for S-AC and S-DC after administration of AC alone (open circle) and after co-administration of cimetidine (filled circle).

Table 4-1. Pharmacokinetic parameters of acebutolol enantiomers after administration of racemate and after co-administration of cimetidine to rats. Data are presented as mean (SD).

Pharmacokinetic parameters	Acebutolol[c]		Cimetidine	
	R-AC	S-AC	R-AC	S-AC
AUC, $\mu g\ h\ l^{-1}$	3155^a (1003)	3007 (895)	2454^a (734)	2367 (705)
$CL_{p.o.}$, $ml\ min^{-1}\ kg^{-1}$	138^a (39)	149 (42)	183^a (55)	189 (54)
$t_{1/2}$, h	1.92 (0.60)	1.86 (0.60)	2.31 (0.72)	2.44 (0.98)
Cl_R, $ml\ min^{-1}\ kg^{-1}$	24.63 (5.56)	25.25 (6.43)	27.41 (10.35)	29.52 (10.75)
Vd / F, $L\ kg^{-1}$	22.70 (8.59)	23.61 (8.55)	27.39 (8.92)	28.92 (10.86)
$\Sigma X_U DC$	165^{ab} (49)	80^b (27)	389^a (140)	248 (109)
$Ae_{0-\infty}$,%	20.15 (10.77)	19.23 (10.60)	15.01 (2.67)	15.63 (2.76)

[a] Significantly different from corresponding enantiomer, $p < 0.05$

[b] Significantly different from corresponding enantiomer for the cimetidine group, $p < 0.05$

[c] Adapted from reference 4

$Ae_{0-\infty}$,%, fraction of dose excreted unchanged in urine.

Disposition of DC was not stereoselective after co-administration of CIM. However, the amount of R-DC recovered in urine, was significantly greater than that of S-DC (R:S = 1.63 ± 0.16) after 24 h post dose. The $AUC_{0-3.5}$ value of DC did not change during combined treatment with CIM. The amount of DC collected in urine however, was increased significantly for both enantiomers.

The mean fraction of AC recovered in urine was approximately 20% after administration of AC alone. A trend towards a decrease in this value was observed after co-administration of CIM for both enantiomers.

DISCUSSION

The results of the present study demonstrated that concomitant CIM administration did not change the concentration-time profiles of AC enantiomers. In fact CIM was co-administered to increase the intragastric pH, as it has been shown that CIM increases the gastric pH which in turn, increases the gastric emptying rate (28). After increasing the gastric emptying rate, the substantial amount of drug emptying into the duodenum soon after administration can cause a rapid increase in plasma concentrations resulting in disappearance of double peaks in the plasma profile of drugs. With AC however, two concentration maxima were retained after CIM administration (Figs. 4-1,4-2). Recently, the double peaks observed in concentration-time profiles of CIM after P.O. administration were found to disappear as the gastric pH was increased (28). In this report the double peaks were observed more frequently when gastric pH was low, however, the double peak disappeared at high gastric pH.

Another area where concomitant administration of CIM with other drugs may play a role is the drug interaction. The change in pharmacokinetic parameters of several agents has been reported in concomitant administration with CIM. To our knowledge, however, there is no report on the interaction between AC and CIM. Our data have shown that the pharmacokinetics of AC were not significantly affected by co-administration of CIM. The absorption rate of AC, however, may have increased since its t_{max} decreased with CIM treatment. This may have been related to elevated gastric pH that increased the concentration of the non-ionized AC moiety, the species that is more readily absorbed than the ionized form. Nonetheless, our results are in agreement with those of others who have seen a decrease in t_{max} of nadolol, a β-adrenoceptor blocker, after concomitant administration with CIM (32).

The most widely known effect of CIM is the inhibition of hepatic metabolism mediated by cytochrome P-450, the reduction in liver blood flow, and inhibition of the proximal tubular secretion of organic cations (33). Co-administration of CIM did not change the AUC of AC or its metabolite, DC, indicating that CIM did not inhibit the metabolism of AC. In fact, if the inhibition of AC had occurred in this sample of rats we should see the increase in AUC of AC as well as a decrease in DC concentrations. By contrast, we observed a trend towards a decrease in the AUC of AC. Furthermore, the amount of metabolite recovered in the urine after 24 h slightly, but significantly increased after co-administration of CIM. This finding may be explained by induction of rat hepatic enzymes by CIM (34,35).

AC is metabolized primarily by hydrolysis of its butyramide group. This gives the primary amine, acetolol, which then undergoes N-acetylation to produce DC. The differences in the metabolism of AC between treatments may be linked to induction of either hydrolysis or acetylation pathways. The metabolism of isoniazid (36) and procainamide (37), drugs that also undergo acetylation, has been investigated by co-administration of CIM. Although renal clearance of procainamide was significantly reduced by CIM, there was no evidence of altered acetylation. Nevertheless, Sevensson *et al.* (38) have reported that CIM did not induce the N-acetyl transferase activity responsible for drug acetylation in rats. We therefore, did not expect CIM to have influenced AC metabolism by alteration of acetylation pathway. The hydrolysis stage however, remains a possibility which could be responsible for these differences. In addition to enzymatic hydrolysis of AC, an increase in the gastric pH induced by CIM perhaps may influence its hydrolysis resulting in elevation of the concentration of intermediate metabolite, acetolol. Nonetheless, an increase in acetolol concentrations could result in elevated DC concentrations which in turn were observed in urine (Table. 4-1). In fact, the conversion of AC to acetolol either in the gastrointestinal tract or after first pass through the liver could result in decreasing the amount of available AC in plasma. Interestingly, a trend toward a decrease in percent of AC recovered in urine which is a reflection of bioavailability, was observed after CIM co-administration which is consistent with the relative availability of 70% that was estimated after CIM co-administration. A decrease in availability produced by CIM could affect the $CL_{p.o.}$ as well as V_d / F . We found a trend towards an increase in $CL_{p.o.}$ as well as V_d / F for AC after this treatment (Table 4-1).

Overall, this study reveals that gastric pH had no influence on appearance of the double peaks phenomena observed after P.O. administration of AC. The rate of absorption of AC however, was influenced after co-administration of CIM, most likely due to increase in pH. This appears to be clinically unimportant as we have found that the extent of absorption is not changed significantly. No other important interactions were observed, thus no special precautions would seem necessary if AC is taken with CIM.

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# ✱CHAPTER 5✱

A Double-Peak Phenomenon in the Pharmacokinetics of Acebutolol Enantiomers after Oral Administration: Discontinuous Absorption of Acebutolol

INTRODUCTION

Acebutolol (AC) is a cardioselective β-adrenergic blocking agent which is used orally for treatment of hypertension, suppression of premature ventricular contractions and other cardiac arrhythmias (1,2). It is a chiral drug and after P.O. administration of racemate its concentration time profiles exhibit two maxima for both R- and S-enantiomer in rats (3-5).

Mechanisms involved for the occurrence of double peaks in the plasma profiles of drugs include enterohepatic recycling (6), discontinuous gastrointestinal absorption (7-9), formation of poorly absorbed micelle complex of drug with bile salts (10,11), variable gastric emptying (12,13), gastric pH (14), or reversible metabolism (15). With AC however, it has been reported that enterohepatic recycling is not responsible for the double peaks phenomena (3) observed after P.O. administration of AC. It is a reasonable conclusion as multiple peaks disappear after iv. administration of AC (16). Variable gastric emptying and gastric pH are other possibilities which can cause the appearance of double peaks in concentration-time profile of drugs. In our previous report (5), we investigated these possibilities by co-administration of cimetidine. In this report it was suggested that a change in gastric pH or gastric emptying is not a reason for appearance of these multiple peaks.

Discontinuous gastrointestinal absorption is another area which may be responsible for the appearance of double peak phenomena. As the double peaking phenomenon for AC was only observed after P.O. dosing, it is clear that the gastrointestinal tract must play an important role in generating these phenomena. We therefore investigated the pharmacokinetics of AC enantiomers after administration of drug in different parts of the gastrointestinal tract to examine the existence of an absorption window. Furthermore, we addressed the question whether the intestinal absorption of AC was affected by bile depletion in the gut lumen of rat. The hypothesis that reversible metabolism of diacetolol (DC), a major metabolite of AC, to parent drug may be responsible for this multiple peaking was also tested.

MATERIALS AND METHODS

Chemicals

Racemic AC and the internal standard (IS), pindolol, were bought from Sigma (Illinois, USA). The metabolite, DC (as hydrochloride salts), was obtained from Rhone-Poulenc Rorer Canada Inc. (Montreal, Que, Canada). All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance

Male Sprague-Dawley rats weighing between 210-300 g were used in the study. The rats were housed under standard conditions in the animal unit where the room temperature was approximately 25° C. Rats were deprived of food for about 8 hours prior to, and for two hours following drug administration, with free access to water. During the period when food was withheld the rats were kept in cages with wide screen bottoms to prevent coprophagy. The intrajugular, intra-duodenal (ID), intraileal (II), and bile duct catheters needed in the experiments were inserted under anesthesia the day before the experiments. The animals were anesthetized by intraperitoneal injections of pentobarbital. Each rat had a polyethylene catheter (0.025 in. i.d. × 0.037 in. o.d.; Dow Corning, Midland, MI, USA) inserted into duodenum, ileum or bile duct. The same type of catheter was inserted into the right jugular vein for blood sampling. The catheters were passed under the skin and exteriorized at the back of the neck. In the bile duct cannulated rat the bile was collected in a vial which was installed in a pre-designed jacket on the back of the rat to prevent bile secretion into duodenum.

Design of the animal experiments

AC solution (50 mg kg^{-1}) was administered ID, or II, in two groups of rats. The same dose was administered orally to a bile duct cannulated rat by gavage. DC was administered either as a bolus injection or oral administration to the last group of rats by gavage to evaluate the reversible metabolism of AC. Blood samples were withdrawn from the heparinized catheter placed in the jugular vein at 0, 2, 15, 30, and 45 min. and at 1, 1.5, 2, 2.5, 3.5, 5.5 and 8 h following drug administration to all groups. Between each blood sample collection, 0.25 ml normal saline was administered *via* the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at -20°C until analyzed. Urine was collected and pooled for 24 h following drug administration. Urine samples were kept frozen at -20°C until just prior to analysis.

Measurement of AC and DC in plasma and urine

Concentrations of R- and S-AC and R- and S-DC in plasma and urine were determined by a normal phase HPLC using fluorescence detection. The analytical procedure has been described in earlier papers (17,18).

Pharmacokinetic data analysis

The area under the plasma concentration-time curve (AUC) for each individual rat was calculated by using the linear trapezoidal rule. The area to infinite time beyond the last sample was estimated by dividing the predicted plasma concentration at the last time point C_{last} by the terminal rate constant (β) calculated by regression through at least three data points in the terminal elimination phase. The terminal elimination half-life ($t_{1/2}$) was calculated by 0.693/β. Clearance (CL) was calculated as D/AUC, where D was the enantiomeric dose administered and AUC was the corresponding area under the plasma enantiomer concentration-time curve. Volume of distribution (Vd/F) was calculated by dividing corresponding CL by elimination rate constant. As the urinary excretion of AC is virtually complete within the first 24 h after single dose administration, renal clearance (CL_R) of each enantiomer was estimated by dividing the cumulative 24-hour urinary excretion of each enantiomers by the corresponding $AUC_{(0-\infty)}$ value. The bioavailabilities of the duodenal and ileal doses of AC enantiomers were determined from the ratio of the AUCs after the duodenal or ileal and iv doses. The AUC of the iv doses was taken from Ref. 16. The plasma AC concentration-time profiles were individually inspected to determine the maximum plasma drug concentration (C_{max}) and the time of occurrence (T_{max}) for each rat studied.

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of enantiomers after administration of AC in different parts of gastrointestinal tract and oral doses of racemate were made by one-way ANOVA followed by Benferoni *post hoc test*. Assumptions of homogeneity of variance were tested using the Levene test prior to ANOVA analysis. Comparisons between the S- and R-AC pharmacokinetic parameters within each study group were assessed utilizing a two-tailed student's t-test for paired data. In all tests, a probability level of significance was pre-set at

$\alpha=0.05$. All results are presented as mean values and standard deviations unless stated otherwise.

RESULTS

Pharmacokinetic parameters of R- and S-AC after ID and II administration of AC as well as P.O. administration in a bile duct cannulated rat are presented in Table 5-1. The pharmacokinetic parameters after oral administration of AC were taken from reference 4 are also shown in Table 5-1.

Table 5-1. Pharmacokinetic parameters of acebutolol enantiomers after oral administration of racemate in different sites of gastrointestinal tract in rats. Data are presented as mean (SD).

Pharmacokinetic parameters	Oral[d]		Duodenal		Ileal		Bile duct	
	R-AC	S-AC	R-AC	S-AC	R-AC	S-AC	R-AC	S-AC
AUC, $\mu g\ h\ l^{-1}$	3155[a]	3007	6113[b]	6000[b]	4123	4080	4742	4550
	(1003)	(895)	(512)	(1074)	(702)	(678)		
CL, $ml\ min^{-1}\ kg^{-1}$	138[a]	149	69[c]	71[c]	103	104	88	92
	(39)	(42)	(6)	(14)	(15)	(15)		
$t_{1/2}$, h	1.92	1.86	1.56	1.44	2.59	2.79	1.94	1.86
	(0.60)	(0.60)	(0.20)	(0.05)	(0.87)	(0.91)		
Cl_R, $ml\ min^{-1}\ kg^{-1}$	24.63	25.25	22.03	24.26	17.04	18.06	18.64	21.63
	(5.56)	(6.43)	(1.1)	(1.42)	(3.31)	(3.45)		
Vd / F, $L\ kg^{-1}$	22.70	23.61	9.34[b]	8.22[b]	23.10	25.18	14.76	14.75
	(8.59)	(8.55)	(2.00)	(1.61)	(9.08)	(9.65)		
$\Sigma X_U DC$	165[ab]	80[b]	854[ab]	560[b]	282[a]	156	506	285
	(49)	(27)	(269)	(219)	(84)	(41)		
T_{max}	1.17	1.17	1.67	1.67	0.21	0.26	0.25	0.25
	0.86	0.86	1.04	1.04	0.09	0.15		
C_{max}	2343	2270	1686	1715	2220	2114	2636	2651
	2079	2090	206	151	774	744		
$Ae_{0-\infty}$,%	20.15	19.23	32.32[b]	34.75[b]	16.62	17.40	21.21	23.63
	(10.77)	(10.60)	(3.31)	(5.07)	(2.41)	(2.62)		

a. Significantly different from corresponding enantiomer, $p < 0.05$; b. Significantly different from corresponding enantiomer for the oral and ileal group, $p < 0.05$; c. Significantly different from corresponding enantiomer for the oral group $p < 0.05$

[d] Adapted from reference 4

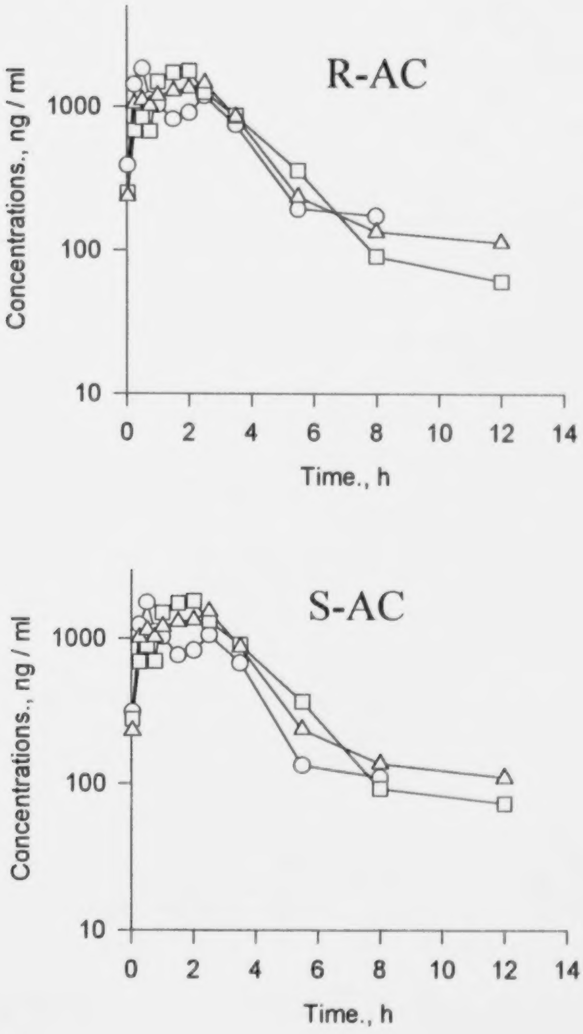

Figure 5-1. Individual plasma concentration of AC enantiomers following duodenal administration of AC (50 mg kg^{-1}) to three rats.

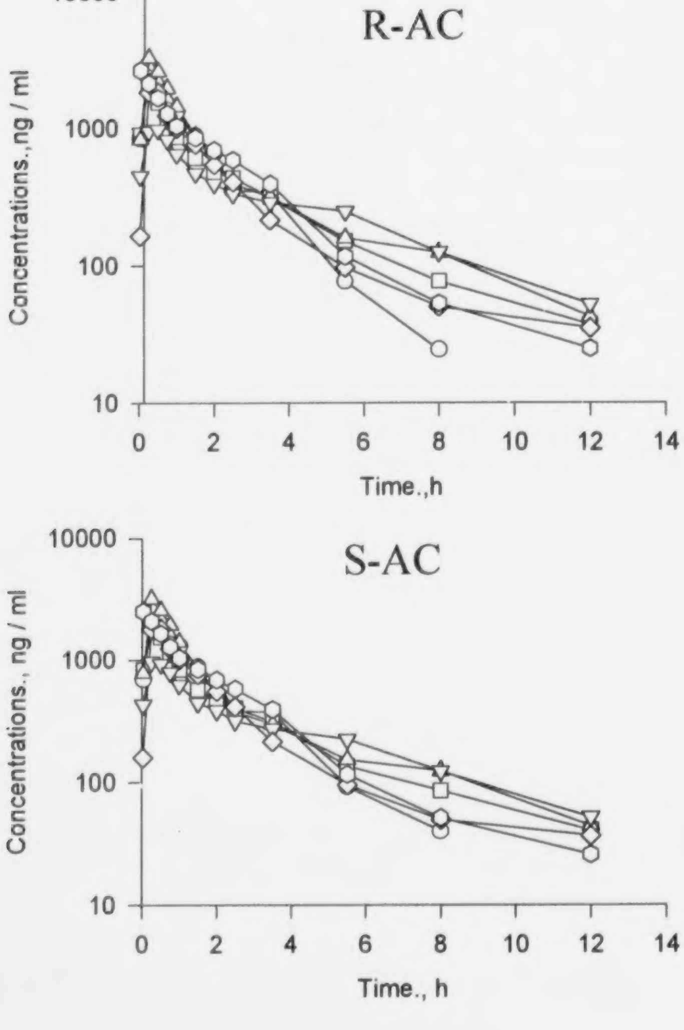

Figure 5-2. Individual plasma concentration of AC enantiomers following ileal administration of AC (50 mg kg^{-1}) to six rats.

The individual enantiomer plasma concentration-time curves for AC enantiomers after ID administration are shown in Fig 5-1. The first peak was achieved during the first 30 min. and the second, was observed after approximately 2-2.5 h. The individual enantiomer plasma concentration-time curves for AC administered into the ileum are shown in Fig 5-2 for both enantiomers. These profiles clearly depict that the double peaking disappears from the plasma profiles after II administration of AC for both enantiomers.

From inspection of the respective $AUC_{0-\infty}$ the extent of AC absorption after ID administration was significantly greater ($p = 0.05$) than those after either oral or ileal administration. Although the absorption of AC after II administration was generally greater than that after oral administration, the difference was not statistically significant. The CL/F values were decreased after both ID and II administration of AC as compared with oral dosing. The differences were, however, significant only after duodenal administration. The mean bioavailability based on the AUC method increased from 36% and 34% for oral dose to 47% and 46% for ileal and 69% and 68% for duodenal dosing for R- and S-AC respectively (Table. 5-1). No significant differences between these treatments for CL_R or $t_{1/2}$, were observed.

A direct comparison of the T_{max} and C_{max} data after drug administration at different sites was complicated by double-peaking in the AC plasma profiles after oral and ID administrations. The T_{max} and C_{max} reported in Table 5-1 refer to the times and concentrations at which the peak plasma concentrations occurred irrespective of whether it was the first or second peak.

The $AUC_{0\text{-}5.5}$ of DC after ID administration was greater than that after either P.O. or ileal administration of AC. However, this difference was significant only after P.O. administration for both enantiomers. The amount of R-DC recovered in the urine was significantly greater than that of S-DC in urine after 24 h post dose for all treatments.

The plasma concentration-time profiles of AC enantiomers after oral administration of racemate in a bile duct cannulated rat is presented in Fig 5-3.

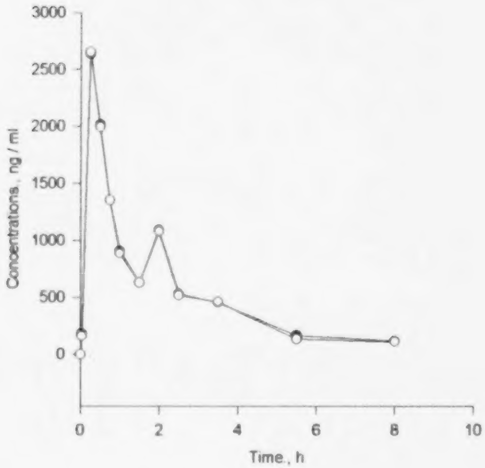

Figure 5-3. Plasma concentration of R- (filled circle) and S-AC (open circle) after oral administration (50 mg kg^{-1}) of racemate in a bile duct cannulated rat.

These profiles clearly depict the double peaking observed in the plasma profiles of R- and S-AC in a bile duct cannulated rat.

After either oral or iv administrations of DC, we did not detect AC either in blood or in urine samples indicating no reversible metabolism for AC enantiomers.

DISCUSSION

Peritoneal surgery and cannulation of different sites of intestine are common means for determining the absorption profile of drugs when administered at different sites of gastrointestinal tract. In these studies, a solution of drug is typically injected into a particular region of the gut through a cannula in laboratory animals. The comparison of pharmacokinetic parameters including the absorption profile of the drug then is determined by assessing these parameters. This method of evaluating the absorption of drug has also been validated for determining the double peak phenomena observed after oral administration of drugs (10). Because the goal of this study was to determine the mechanism which generates the double peak phenomena and it has been reported (3) that rat is a good animal model for such studies, we chose the rat to do this study.

AC is a beta-blocker with erratic absorption in both man (19-24) and rat (3,4). A previous study of AC has shown that AC is rapidly absorbed and the concentration-time profile of its enantiomer depicts the double peak phenomena after oral administration of racemate in rat (4). This is not due to enterohepatic recirculation (3), gastric pH or a variable gastric emptying (5), however, the window of absorption and poorly absorbed micelle formation or reversible metabolism has been suggested as the possible mechanisms of this phenomenon (3-5).

Various models of discontinuous gastrointestinal absorption have been developed to describe the multiple peaks seen after oral administration of drugs (7-9,25). Among those models, the existence of a classical absorption window which is incorporated with a non-absorbing gastrointestinal segment between one or more absorption sites (7,9,25) is the more probable model which may explain our observations. Pharmacokinetic analysis of the data demonstrate that extent of absorption from the duodenum was significantly greater than that from ileal administration based on comparison of AUCs after two treatments. This data is consistent with the absorption window theory. This theory proposes that a drug may be absorbed largely from a specific area within the proximal gastrointestinal tract (i.e. duodenum), resulting in the initial peak plasma concentration. A second absorption window more distal in the gastrointestinal tract (i.e., ileum or colon) may absorb some of the remaining drug and produce a smaller, second absorption peak 2 or 3 hr later. The specific site of absorption through the gastrointestinal tract for AC may be in duodenum as the capacity for AC absorption is greatest in the upper regions of the small intestine. This conclusion is readily defended on grounds of increased absorptive surface area of the duodenum relative to ileum (26), and its ability to solubilize poorly soluble compound through bile salts solubilization and related mechanisms (27).

It was found that duodenal administration of AC had no effect on double peaks in the plasma concentration time profile (Fig 5-1) which also confirms our previous results that gastric emptying has no effect on generating double peaks (5). Instead, the double peaks disappeared following ileal administration of AC (Fig 5-2) confirming the hypotheses that absorption window plays an important role in generating the double peak phenomena in plasma profiles of AC enantiomers. Similar results have been reported for the double peaking phenomena apparent after oral dosing of veralipride (7).

Because of the lower $AUC_{0-\infty}$ after oral administration of AC as compared with ID administration, it is apparent that the stomach plays a role in the absorption process of AC even though it is unlikely to be a direct effect. Differences between the extent of absorption and the bioavailability of AC after oral administration as compared with duodenal or ileal administration may suggest that the absorption of AC in gastrointestinal tract is pH related. After oral administration of AC, it may hydrolysis in acidic pH of stomach, thus it is less available to be absorbed. Consequently, the bioavailabilities of both enantiomers after oral administration are lower than those after duodenal or ileal administration. The question may arise of why there is a difference in extent of absorption and bioavailability after duodenal and ileal administration even though the pH is more basic in ileum than duodenum. This could also be explained by the time that the drug is in contact with the absorpting surface. As the time that a drug resides in absorptive surface after duodenal administration is more than that after ileal administration, the extent of absorption could be increased.

A trend towards an increase in $AUC_{0-5.5}$ of DC after duodenal administration, which is a reflection of increased AC availability, was observed, however, it failed to reach a significant level. Consistently, a trend towards an increase in the amount of AC and a significant increase in the amount of DC recovered in the urine were observed. A higher recovery of AC and DC in urine after duodenal administration may account for the finding that systemic availability of duodenal administration was 69% and 68% compared to 0.47% and 0.46% after ileal and 0.36% and 0.34% after oral administration of AC for R- and S-enantiomer, respectively.

Another reason which could describe the multiple absorption peaks is the formation of poorly absorbed micelle complex of drug with bile salts. It has been suggested that double peaks observed after oral administration of pafenolol is due to this phenomenon (10,11). The authors described the initial pafenolol peak as being formed after rapid micelle formation in the small intestine resulting in cessation of absorption. Dissociation of the micelles in the ileum due to active reabsorption of bile salts results in the second peak. We also tested this hypothesis to see whether the same process could cause multiple peak phenomena after oral administration of AC. Following oral administration of AC to a bile duct cannulated rat the double peak phenomena remained (Fig 5-1). Consequently, no further rats were studied.

It has been suggested that reversible metabolism could play a role in generating the multiple peak phenomenon in a way that metabolites of a drug could be returned back to the parent drug either by gastrointestinal bacteria or by enzymes. We administered DC, the major metabolite of AC to see whether reversible metabolism is occurred. After either oral or iv administrations of DC the parent drug, AC, was not detected either in plasma or urine samples. Therefore, it was concluded that reversible metabolism does not contribute to appearance of double peaking in plasma profile of AC.

In conclusion, our study demonstrates that duodenum may be the main site for the absorption of AC after oral administration which is probably the underlying mechanism for double peak phenomena observed after oral administration of drug.

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## ❋CHAPTER 6❋

Pharmacokinetics of Metoprolol Enantiomers Following Single and Multiple Administration of Racemate in Rat

INTRODUCTION

Hypertension is one of the major risk factors associated with heart disease. In the therapy of hypertension different drugs with different mechanism of actions are used. β- adrenoceptor blocking agents are now widely accepted as first line drugs in the treatment of hypertension and angina (1). Among them metoprolol (MET Fig 6-1) is a cardioselective β-blocker which is marketed as a racemate mixture. A moderate enantioselectivity of MET pharmacokinetics has been reported in human (2). Furthermore, the pharmacokinetic studies with metoprolol to investigate the effect of age (3,4) and to explain the pharmacokinetics and pharmacodynamics of racemic MET have been described (5). Although non-stereospecific studies suggest accumulation of metoprolol after multiple dose administration in humans (6), the pharmacokinetic characteristic of MET enantiomers after multiple dosing has not been fully investigated. Consequently, they have not accounted for potential enantioselectivities in pharmacokinetic processes.

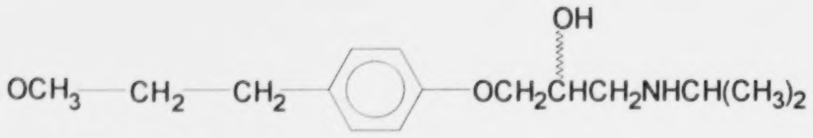

Figure 6-1. Chemical structure of racemic metoprolol

The importance of performing multiple-dose studies in assessing drugs, including β-blockers, has been suggested (6). Moreover, no studies are available dealing with the pharmacokinetics of MET enantiomers after multiple dosing in rats or in humans. We therefore sought to study the pharmacokinetic characteristics of MET enantiomers after single and multiple oral administration of racemate in rat model. Because, the rat is a commonly used animal to study the pharmacokinetic, pharmacology and toxicology of enantiomers we decided to use this species.

MATERIALS AND METHODS

Chemicals

Metoprolol tartrate was obtained from Sigma Chemical Company (Illinois, USA). The internal standard (IS), (+)-naproxen chloride, was synthesized in our laboratory by reacting thionyl chloride with (+)-naproxen and purified by repeated re-crystallization. Thionyl chloride and (+)-naproxen were obtained from Aldrich Chemical Co.(Milwaukee, WI, USA). All other chemicals and reagents were HPLC or analytical grade.

Surgery and animal maintenance

Male Sprague-Dawley rat weighing between 245-285 g were kept on a light-dark cycle of 12 hours at a room temperature of 25° C. They were fasted for about 8 hours before and two-hours following drug administration for the single dose study and also before the last dose of multiple dosing, with free access to water. Animals were catheterized with silastic tubing (0.025″ i.d. × 0.037″ o.d.; Dow Corning, Midland, MI, USA) at the right jugular vein while they were under general anesthesia with pentobarbital administered via the peritoneal route. The animals were allowed to recover overnight prior to the experiments. During this time the animals were individually stored in 18″ × 9.5″ × 8″ polycarbonate rodent cages.

Dosing and sample collection

Racemic MET dissolved in normal saline was administered in doses of 20 mg kg^{-1} (one ml/kg) either as single or as multiple doses (four times per day for four days) by gavage. Blood (250 μl) was collected from the jugular vein cannula just prior to and at 2, 10, 20, 30 and 45 minutes and 1, 1.5, 2, 2.5 and 3.5 h after drug administration. Between each blood sample collection 250 μl normal (0.9%) saline was administered via the jugular vein cannula as fluid replacement and the cannula was heparinized (10 U ml^{-1}). Blood samples were immediately centrifuged and the plasma was separated and immediately frozen at -20°C until analyzed.

Stereospecific HPLC assay of MET

Concentrations of R- and S-MET in plasma were determined utilizing a previously reported stereospecific HPLC method (7). Briefly, samples were extracted with a mixture of methyl tertiary butyl ether (MTBE) : isooctane (75:25, v/v), after addition of (+)-naproxen chloride as the internal standard and subsequent alkalization with 1 M sodium hydroxide. After the extract was evaporated, the resulting residues were reconstituted in mobile phase and injected in to the HPLC. The enantiomers were separated at ambient temperature on a commercially available 4.6 x 250-mm amylose carbamate-packed chiral column (Chiracel OD-H) and chromatographed via normal phase HPLC using fluorescence detection.

Pharmacokinetic data analysis

The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. The area from the last concentration point (C_{last}) to infinity was calculated as C_{last}/β, where β was the terminal elimination rate constant calculated by regression through at least three data points in the terminal elimination phase. The terminal elimination half-life ($t_{1/2}$) was calculated by $0.693/\beta$. Oral clearance ($Cl_{p.o.}$) was calculated by dividing the total administered enantiomeric dose with the AUC after oral administration ($AUC_{p.o.}$). Maximum plasma concentrations (C_{max}) and the time for maximal concentrations to be reached (T_{max}) were derived from graphical analysis of plasma MET concentrations *vs.* time. Volume of distribution (V_d/F) was calculated by dividing corresponding $Cl_{p.o.}$ by β.

Statistical analysis

Statistical comparisons of the pharmacokinetic parameters of MET enantiomers either after single or after multiple dose administration of MET were assessed utilizing a two-tailed student's t-test for paired data. Comparisons of R- *vs.* R and S *vs.* S-MET after single and multiple dose administration were made by utilizing a two-tailed test assuming equal variance. In all tests, a probability level of significance pre-set at $\alpha = 0.05$. Results are expressed as mean ± SD.

RESULTS

Single dose administration

Average plasma concentration of MET enantiomers after single dose of racemate are depicted in Fig 6-1. Average maximum plasma concentrations of MET during the single interval were 235 ± 138 μg/l for the R- and 227 ± 132 μg/l for the S-enantiomer. These values were obtained approximately 12 minutes after dosing the rats. The terminal half-lives were about 35 min for both enantiomers. The mean $AUC_{0-\infty}$ value of the R-enantiomer was higher than that of S-enantiomer (97 versus 83 $\mu g\ h\ l^{-1}$). Consequently, the average oral clearance were greater for S-MET (2.66 ± 0.85) than that of R-MET (1.99 ± 0.87).

Multiple oral administration

The average plasma-concentration versus time curves of MET enantiomers are illustrated in Fig 1. The average C_{max} values of the MET enantiomers reached 285 ± 142 μg/l and 289 ± 151 μg/l for R- and S-MET, respectively. The terminal half-lives did not significantly change upon repetitive dosage. The mean plasma enantiomeric concentrations of MET were increased significantly than the corresponding $AUC_{0-\infty}$ after single oral dose (Fig. 6-1). Consequently, the $CL_{p.o.}$ were higher after single dose administration.

Table 6-1. Pharmacokinetic parameters of metoprolol enantiomers after administration of racemate to rats. Data presented as mean, (SD).

Pharmacokinetic parameters	Single dose		Multiple dose	
	R	S	R	S
$^{a}AUC_{0-\infty}$, μg h l^{-1}	$97^{*\#}$ 38	83 29	309^{*} 95	295 109
$CL_{p.o.}$, L min^{-1} kg^{-1}	$1.99^{*\#}$ 0.87	2.26 0.85	0.59^{*} 0.21	0.64 0.26
$t_{1/2}$, min.	35 11	35 13	33 9	32 6
V_d / F, L kg^{-1}	63 19	76 20	40 25	39 24
Rat weight, g	261 12		277 9	

[*] significantly different from corresponding enantiomer, $p<0.05$

[#] significantly different from corresponding enantiomer after multiple dose, $p<0.05$

a. $AUC_{0-\tau}$ after multiple dosing

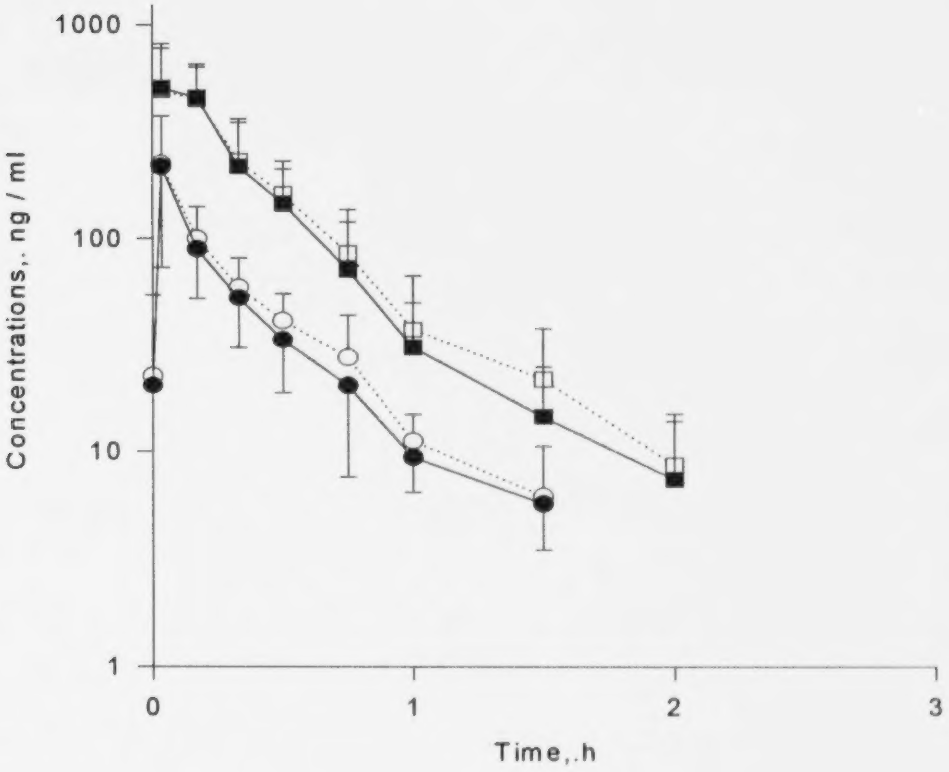

Figure 6-2. Average plasma concentration versus time profiles for R- and S-MET after administration of the racemate as a single or multiple doses. R-MET after single dose = open circles; S-MET after single dose = filled circles; R-MET after multiple dosing = open squares; S-MET after multiple dosing = filled squares; error bars represent SD.

DISCUSSION

Single dose administration

Plasma concentration-time profiles (Fig. 6-1) indicate that the pharmacokinetics of MET is stereoselective in rat after oral administration of racemate. Slightly but significantly greater amounts of the R-enantiomer of MET were seen in plasma after a 20 mg kg^{-1} P.O. dose. Consequently, the Cl_{po} were higher for S-enantiomer as it compared with R-MET. MET undergoes hepatic first-pass extraction and its metabolism is related to debrisoquin oxidation phenotype (8). The higher Cl_{po} of S-MET may be due to its higher intrinsic clearance, as MET is a low protein bind drug (9) and no differences in free fraction between two enantiomers are expected. Our results are in agreement with the recent report on the enantiomeric disposition of MET in different age groups in rat (4).

MET is rapidly absorbed and C_{max} was reached at 12 min post dose for both enantiomers. Further, the estimated average $t_{1/2}$ of the enantiomers were similar (35 min) for both R-and S-MET. This indicates that Sprague-Dawley rat is an extensive metabolizer of MET as it has been reported that the half life of R-MET is longer than that of S-MET in poor metabolizers, whereas no differences is observed in extensive metabolizers (10) of MET. Therefore, rat appears to be a good animal model as the majority of Caucasians are "extensive metabolizers" of MET.

Multiple dosing

The average plasma-concentration versus time curves of MET enantiomers after single and multiple dose administration (Fig. 6-1) show that the plasma concentrations at study state were greater than predicted by the single dose data. This is indicated by the comparison of the mean area under the plasma concentration time curve for the single dose and the dosage interval areas during multiple dosing (Table 6-1). The plausible explanations for this increase in the AUC during multiple dosing are 1) a decrease in systemic clearance of MET during multiple treatment because of potential reduction in hepatic blood flow and/or 2) a decrease in pre-systemic clearance of MET upon repeated administration of drug due to saturation of first pass effect. As MET is a low protein binding drug (10%) a change in clearance due to a change in V_d is not expected. Furthermore, the $t_{1/2}$ of MET is not changed after chronic administration as compared with single dose treatment therefore, a decrease in systemic clearance upon multiple dose administration of MET is unlikely.

Another explanation for greater value for AUC during multiple dosing is the saturation of first pass-effect due to repeated administration of MET, which leads to a higher systemic availability of enantiomers. Nonetheless, other investigators have also found that the fraction of the dose available systemically after long term treatment has increased in human (13,14). The 26% and 10% increase in AUC after multiple dosing have been reported by these authors. Furthermore, marked effects of multiple dosing on bioavailability of metoprolol have been reported by Kendall *et al.* (6) and collste *et al.* (15). In these two studies the mean increase in AUC was 41% and 57% after treatment with metoprolol 100 mg twice daily for 8 days and 6-12 weeks, respectively. Our results, however, are more consistent with Myers *et al.* (12) report in which 148% increase in the AUC during multiple dosing with MET 50 mg 3 times daily for at least 3 weeks has been reported. An increase in AUC during long term treatment for other β-blockers including acebutolol have also been reported (11).

In conclusion, this study illustrates the importance of considering multiple dose studies in assessing the pharmacokinetics of MET enantiomers. Further, we found that rat may be a good animal model for studying the pharmacokinetics of MET.

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## ✱CHAPTER 7✱

Influence of Acebutolol and Metoprolol on Cardiac Output and Regional Blood Flow in Rats

INTRODUCTION

Beta adrenergic receptor blocking drugs are now widely prescribed for both angina and hypertension. Although these drugs are equally effective in reducing blood pressure, they are different in ancillary properties such as affinity for β_1- and β_2-adrenoceptors, bioavailability, lipid solubility and partial agonist activity or intrinsic sympathomimetic activity (ISA, Table 1-1). These ancillary properties, however, may be important with regard to safety, side effects, and the hemodynamic effects of these drugs.

In particular, AC and MET are β blockers whose properties are similar in most aspects, (Table 1-1). Both AC and MET are cardioselective, low protein bound drugs with the pK_a of 9.7 and the oral bioavailability of approximately 40-50%. MET is eliminated from the body by hepatic metabolism. AC however, is eliminated by both renal clearance and hepatic metabolism. Although these agents are similar in most pharmacokinetic or pharmacological activity in lowering the blood pressure they are different in possession of some ancillary properties such as ISA. While AC possesses some degree of ISA MET is devoid of this property.

The presence of ISA in β blockers modifies the pharmacological effects of these agents. It has been reported that cardiac hemodynamics and the effects of peripheral circulation vary according to the extent of ISA possesses by β blockers (4). Drugs with ISA cause less decrease in heart rate and cardiac output (CO) at rest than those without it. Further, the effects of ISA may be different after acute administration of drugs as it compared with the chronic administration (8).

We sought to study the acute and chronic hemodynamic effects of AC with moderate degree of ISA and MET, which is devoid of ISA in rat to demonstrate CO and regional blood flow (RBF) changes induced by beta blockers with different degrees of ISA. To determine values of CO and RBF the radioactive microsphere method was used.

MATERIALS AND METHODS

Chemicals

AC hydrochloride and MET tartrate were obtained from Sigma Chemical Company (Illinois, USA). Radio-labeled microspheres (^{141}Ce and ^{85}Sr) with a diameter of 15 μm were purchased from Dupont NEN (Wilmington, DE, USA) and dissolved in physiological saline containing 0.01% Tween 80. All other chemicals and reagents were analytical grade.

Surgery and animal maintenance

Male Sprague-Dawley rat weighing between 310-360 g were kept on a light-dark cycle of 12 hours at a room temperature of 25° C. Under general anesthesia with pentobarbital administered via the peritoneal route, the animals were catheterized with silastic tubing (0.025 in. i.d. × 0.037 in. o.d.; Dow Corning, Midland, MI, USA) at the right jugular vein for drug administration. A cannula, the top of which was tapered to reduce damage to cardiac tissue, was inserted into the left ventricle through the right carotid artery. The femoral artery was cannulated to allow for taking reference blood samples.

Dosing and sample collection

The CO and RBF were measured with the microsphere method (Fig 7-1). The cannula for the femoral artery was connected to the heparinized syringe (1.0 ml) fixed in a withdrawal pump (syringe infusion pump, Harvard Apparatus, Millis, Massachusetts, U.S.A). Microspheres labeled with either ^{141}Ce or ^{85}Sr were suspended in 0.5 ml of normal saline which were injected into the left ventricle over an interval of 30 seconds through a cannula connected to left ventricle followed by 0.4 ml physiological saline to flash the microspheres remaining in the cannula. Ten seconds before the administration of microspheres, reference blood sample withdrawal was begun from the femoral artery at the rate of 0.26 ml min^{-1}. The microspheres were injected and flushed with 0.4 ml of physiological saline. The withdrawal of reference blood sample was stopped approximately after two minutes. Before the injections of drug or control a microsphere labeled with different nuclides were administered randomly as described above. Again, one minute and ten minutes after the administration of either drugs or control the second microspheres labeled with the second nuclide were administered, and rat was killed by intravenous administration of pentobarbital (50 mg kg^{-1}). The heart, lung, liver, stomach, spleen, muscle, kidneys, skin, and brain were sampled, blotted, and weighed. The radioactivities were measured by a gamma counter (Miniaxi γ Canberra packard, Canada).

For evaluating the long term effects of AC or MET, rats were administered either drugs or control orally every eight hour for four days. AC (10) and MET (11) are rapidly absorbed after oral administration in rat. Thus, the last dose was administered and it was allowed to absorb for ten minutes. Rat was anesthetized, cannulated as explained and microspheres labeled with different nuclides were administered randomly. Rat was killed by intravenous administration of pentobarbital, different organs were sampled, blotted, weighed and radioactivity in each organ was measured as described above.

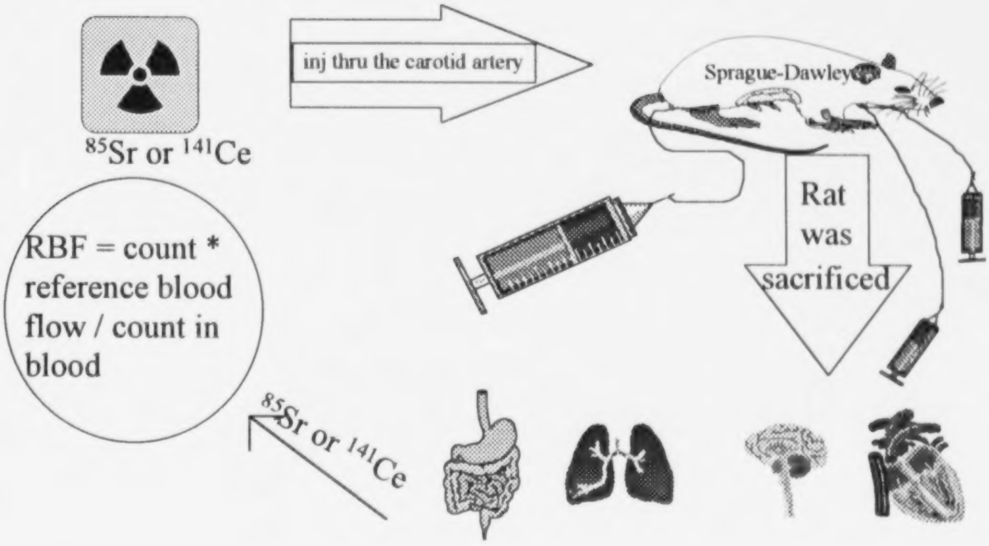
inj thru the carotid artery
Sprague-Dawley
^{85}Sr or ^{141}Ce
RBF = count * reference blood flow / count in blood
Rat was sacrificed
^{85}Sr or ^{141}Ce

Figure 7-1. Cardiac output and blood flow measurements by radioactive microspheres.

CO and RBF measurements

Tissue activity of each isotope was calculated after subtraction of the background and the overlapping isotope activity by conventional stripping procedures (12). For computation of the counts injected into the left ventricle, the initial activities in the vial from which the microspheres were aspirated were counted. The CO and the RBF were computed as follows:

$$\text{CO (ml min}^{-1}\text{ kg}^{-1}) = \frac{\text{Counts injected in left ventricle} * \text{aortic withdrawal flow (ml / min)}}{\text{Counts in aortic blood} * \text{Rat wt, kg}}$$

$$\text{RBF (ml min}^{-1}\text{)} = \frac{\text{organ count * aortic blood withdrawal flow (ml / min)}}{\text{counts in aortic blood}}$$

$$\text{RBF (ml min}^{-1}\text{ g}^{-1}\text{)} = \frac{\text{organ blood flow (ml / min)}}{\text{organ wt, g}}$$

Statistical analysis

The effects of drugs or control on CO and RBF in different organs were evaluated by comparing drug or control treatment with the baseline utilizing a two-tailed student's t-test for paired data. The baseline values for CO and RBF after different treatments were compared by using one way analysis of variance (ANOVA). Comparisons of changes in CO and RBF after AC or MET administration to control values in an organ were made by using a one way ANOVA. In all tests, a probability level of significance pre-set at $\alpha = 0.05$. Results are expressed as mean ± SEM.

RESULTS

For better understanding and to help to interpret the results in our study, the hemodynamic events can be arbitrarily divided into three stages: 1) one minute after administration of drugs or control; 2) 10 minutes after administration of drugs and control; and 3) after chronic administration of drugs and control. The values of CO and RBF after saline administration did not differ in control group (Table 7-1) in any stages as it was compared with the baseline values.

There were no significant differences in baseline values either after drugs or after control treatments. The effects of AC and MET on CO and RBF in all stages are summarized in Tables 7-2 and 7-3. Both drugs reduce the CO significantly in both stages one and two as it compared with the baseline values. The percent of change in CO after administration of either drug however, is significantly different only in stage one as it compared with the same values in control group (Figs 7-2,7-3).

Table 7-1. The acute effect of intravenous administration and the chronic effect of oral administration of saline, as control, on cardiac output and regional blood flow in Sprague-Dawley rats. Data are presented as mean ± SEM.

Organs ml/min/g	Baseline	1 min	% Change	Baseline	10 min	% Change	Chronic
Liver	0.15 ± 0.03	0.13 ± 0.03	15.9 ± 5.6	0.15 ± 0.02	0.12 ± 0.02	10.8 ± 3.9	0.18 ± 0.01
Heart	3.92 ± 0.74	2.88 ± 0.46	21.6 ± 6.2	2.74 ± 0.28	2.14 ± 0.55	20.8 ± 6.2	2.44 ± 0.14
R-Kidney	3.84 ± 0.41	3.02 ± 0.35	20.4 ± 7.9	4.05 ± 0.21	2.73 ± 0.20	26.2 ± 5.6	2.91 ± 0.26
L-Kidney	3.85 ± 0.40	3.25 ± 0.39	15.4 ± 6.2	3.79 ± 0.35	2.60 ± 0.28	26.9 ± 4.8	3.06 ± 0.36
Spleen	1.80 ± 0.29	1.41 ± 0.19	17.2 ± 9.9	1.53 ± 0.20	1.03 ± 0.15	20.8 ± 3.7	1.04 ± 0.17
Lung	0.91 ± 0.13	0.63 ± 0.18	30 ± 7.2	0.63 ± 0.08	0.38 ± 0.07	39.8 ±6.42	0.38 ± 0.05
Stomach	0.46 ± 0.04	0.35 ± 0.04	22.4 ± 8.9	0.47 ± 0.04	0.28 ± 0.04	22.1 ± 2	0.39 ± 0.11
CO (ml/min/kg)	338 ± 27	304 ± 30	10.6 ± 3.9	276 ± 15	228 ± 23	16.4 ± 8.4	229 ± 22

Table 7-2. The acute effect of intravenous administration and the chronic effect of oral administration of acebutolol hydrochloride on cardiac output and regional blood flow in Sprague-Dawley rats. Data are presented as mean ± SEM.

Organs ml/min/g	Baseline	1 min	% Change	Baseline	10 min	% Change	Chronic
Liver	0.15 ± 0.02	0.08 ± 0.01[a]	48.3 ± 6.2[b]	0.14 ± 0.02	0.12 ± 0.02[a]	19.8 ± 5.8	0.19 ± 0.01
Heart	4.14 ± 0.82	2.06 ± 0.36[a]	45.7 ± 8.6[b]	3.32 ± 0.46	2.29 ± 0.34	41.9 ± 7.7	2.77 ± 0.42
R-Kidney	4.36 ± 0.52	2.54 ± 0.27[a]	38.2 ± 8.0	3.60 ± 0.41	3.12 ± 0.55[a]	43.1 ± 18.4	2.82 ± 0.26
L-Kidney	4.45 ± 0.62	2.73 ± 0.36[a]	34.7 ± 8.7	3.6 ± 0.45	2.99 ± 0.56[a]	35.6 ± 10.0	2.65 ± 0.25
Spleen	1.9 ± 0.36	0.69 ± 0.07[a]	59.3 ± 5.6[b]	1.47 ± 0.13	1.15 ± 0.27[a]	38.3 ± 10.6	0.78 ± 0.08
Stomach	0.45 ± 0.07	0.37 ± 0.06	18.3 ± 6.8	0.43 ± 0.11	0.39 ± 0.10	30.1± 5.1	0.33 ± 0.07
CO (ml/min/kg)	437 ± 49	203 ± 32[a]	53.6 ± 4.8[b]	373 ± 52	283 ± 32	27.6 ± 8.0	260 ± 20

[a] Significantly different than the baseline, $p < 0.05$

[b] Significantly different than the control, $p < 0.05$

Table 7-3. The acute effect of intravenous and the chronic effect of oral administration of metoprolol tartrate on cardiac output and regional blood flow in Sprague-Dawley Rats. Data are presented as mean ± SEM.

Organs ml/min/g	Baseline	1 min	% Change	Baseline	10 min	% Change	Chronic
Liver	0.16 ± .03	0.10 ± 0.02[a]	40.4 ± 7.3[b]	0.17 ± 0.03	0.14 ± 0.03[a]	25.3 ± 2.6	0.23 ± 0.03
Heart	4.00 ± 0.84	2.58 ±0.54	55.3 ± 6.5[b]	3.39 ±0.81	2.07 ±0.48[a]	36.5 ± 7.5	1.50 ± 0.25
R-Kidney	4.11 ± 0.47	2.66 ± 0.37[a]	35.5 ± 6.0	3.91 ± 0.69	2.83 ± 0.41[a]	24.8 ± 5.9	3.43 ± 0.32
L-Kidney	3.83 ± 0.42	2.39 ± 0.29[a]	37.3 ± 5.2	3.82 ± 0.68	2.88 ± 0.37[a]	25.2 ± 3.5	3.20 ± 0.32
Spleen	1.45 ± 0.23	0.84 ± 0.14[a]	47.1 ± 7.5	1.26 ± 0.30	0.68 ± 0.15[a]	42.6 ± 6.2	1.04 ± 0.21
Stomach	0.50 ± 0.07	0.29 ± 0.05[a]	37.1 ± 11.3	0.37 ± 0.04	0.25 ± 0.05[a]	42.8 ± 4.2	0.28 ± 0.03
CO (ml/min/kg)	378 ± 91	148 ± 48[a]	65 ± 8.5[b]	395 ± 97	233 ± 31.	31 ± 8.9	233 ± 12

[a] Significantly different than the baseline, $p < 0.05$

[b] Significantly different than the control, $p < 0.05$

Changes in RBF occurred in parallel with the changes in CO (Tables 7-2,7-3). These values are reduced significantly in most organs as the blood flow is compared after drug administration with the baseline (Table 7-2,7-3) in stages one and two. The percent of change in RBF after either AC or MET administration in stage one is greater than the same values in control group in the liver, heart, lung, kidneys, spleen, stomach and brain, although the differences are significant only in the liver, heart, and spleen (Figs 7-2,7-3).

Following multiple dose administration of either AC or MET there were no significant differences between CO or RBF in any organs as they compared with the control values (Fig 7-4,7-5).

There were no significant differences between the effects of AC and MET on CO and RBF in any stages.

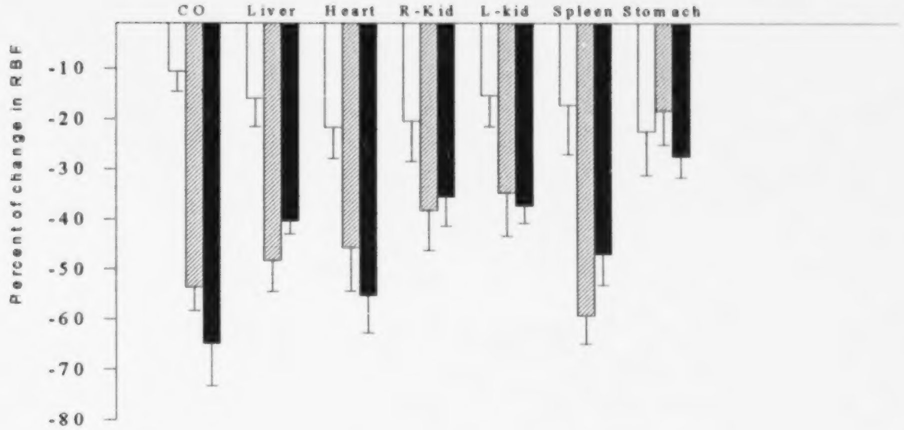

Figure 7-2. Percent of change in CO and RBF one minutes after administration of saline (open bars), AC (hatched bars) or MET (solid bars) in Sprague-Dawley rats. Mean ± SEM

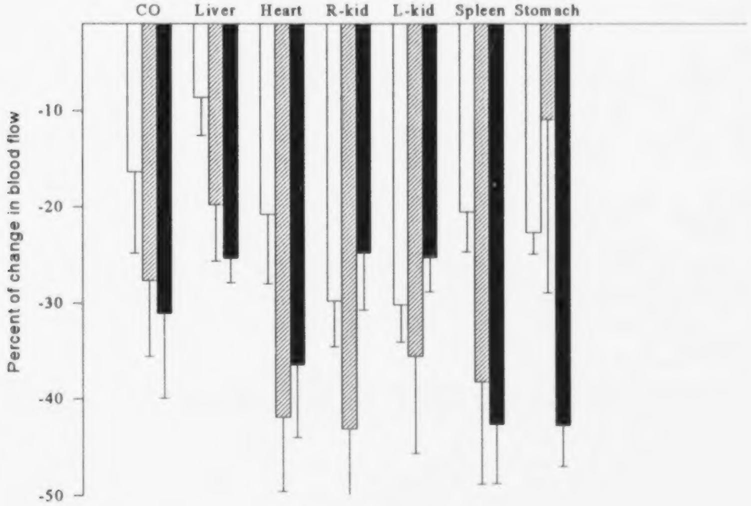

Figure 7-3. Percent of change in CO and RBF ten minutes after administration of saline (open bars), AC (hatched bars) or MET (solid bars) in Sprague-Dawley rats. Mean ± SEM

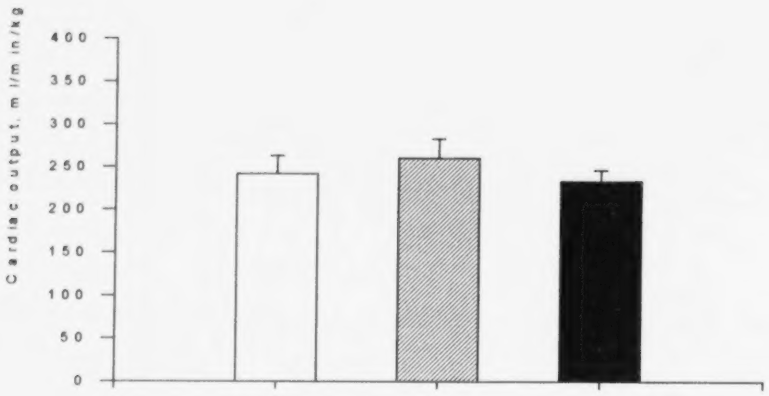

Figure 7-4. CO after four days treatment with saline (open bars), AC (hatched bars) and MET (solid bars) in Sprague-Dawley rats. Mean ± SEM.

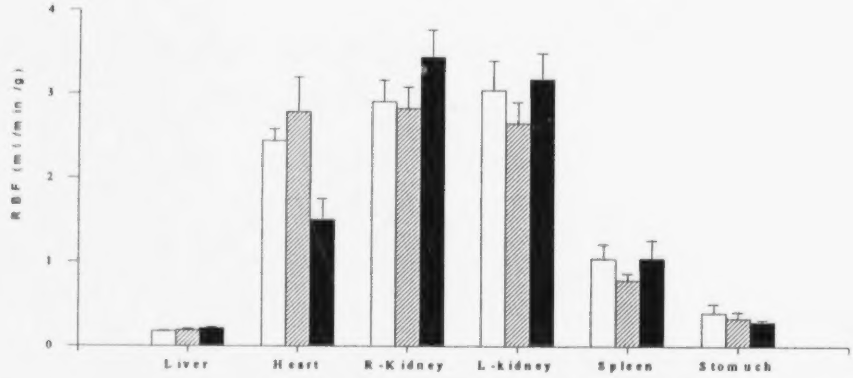

Figure 7-5. RBF after four days treatment with saline(open bars), AC (hatched bars) and MET (solid bars) in Sprague-Dawley rats. Mean ± SEM.

DISCUSSION

The use of microspheres to measure simultaneously total CO as well as RBF has been validated and used in different animal (13-19) including rat (20,22). In this study, the microsphere method was used to evaluate the effects of two different β-blockers with different ancillary properties on CO, and RBF before and after the drug or control administration.

The precision of microsphere technique depends on the number of microspheres trapped in the respective sample. It has been reported (23) that injection of 360 000 microspheres or fewer will not cause any hemodynamic disturbances. Further, to have a reliable result, the number of microspheres in each sample should exceed 200 microspheres per sample to provide a precision of 10% at a confidence level of 95% for rat (24). Thus, the number of microspheres was fixed approximately at 100,000 microspheres so that each sample had more than 200 microspheres. As we have sampled small pieces of muscle and skin in this study therefore, the numbers of microspheres in these samples were less than 200 in each piece. Consequently, we excluded these samples from experiment and subsequent analysis. All other samples contained at least 400 microspheres as determined by specific activity of the microspheres and the radioactivity of each sample. Moreover, four rats were excluded from the final analysis and the experiment was repeated on fresh rats because of catheter malposition evidence for poor microsphere mixing. Even distribution of microspheres to tissue was confirmed by the extent that approximately the same values for blood flow were observed in each kidney before or after drug administration.

Our results compare favorably to the CO and RBF reported by others in rat (25,26). The CO of pentobarbital anaesthetized rat is variably quoted as being from 121 to 388 ml min^{-1} kg^{-1}. Harashima *et al.*(26) recently reported microsphere method which gave an average output of 331 ± 34 ml min^{-1} kg^{-1}, a value essentially the same as obtained in the present study as baseline values (Tables 7-1,7-2,7-3).

The change in CO by two β-adrenoceptor blocking agents after the initial iv doses largely agree with previously reported response to these drugs when administered intravenously (27). A rapid fall in CO, was observed after acute administration of AC and MET. Earlier studies (6,28,29) with iv doses of AC have shown that the magnitude of the fall in CO of this drug is intermediate between that of pindolol with the most degree of ISA and propranolol which is devoid of ISA. In the present study, the percent of fall in CO was 54 ± 5 and 27 ± 8 in stages one and two after administration of AC respectively. These values were increased to 65 ± 9 and 31 ± 9 after MET administration. Although the differences in percent of change in CO between these two agents failed to reach a significant level a trend toward an increase in this value was observed after MET administration. The degree of ISA possesses by AC may explain the present finding. Among β-adrenoceptor blocking agents, pindolol has the most degree of ISA followed by penbutolol, oxprenolol, alprenolol, celiprolol and AC (30-32). As AC has a relatively low degree of ISA among β-blockers therefore, it may not have enough sympathomimetic activity to make a significant difference between this drug and MET with no ISA. Nonetheless, other investigators have also failed to detect any significant differences in hemodynamic changes caused by β-blockers with relatively low degree of ISA as they have compared them with the β-blockers which are devoid of ISA (27). Moreover, Manttari *et al* have also reported that the hemodynamic differences between MET and AC are not statistically significant in hypertensive patients (33).

The change in RBF after acute administration of either AC or MET is striking. In nearly every tissue which was examined, there was a significant decrease in blood flow after either AC or MET administration when the blood flow was compared with the baseline values (Table 7-2,7-3). When the percent of change in RBF is compared with the same value in a control group however, it is significant in the liver, heart and spleen only one minute after drug administration.

The hemodynamic effects of AC after chronic administration have also been reported by others (36,37). In those studies, the hypotensive action of the drug in the long run was accompanied by a decrease in heart rate. The vascular resistance and CO however, return to normal (38). The finding that CO remains unchanged after long term administration of AC is confirmed by the present study. However, the long term antihypertensive response to β-blockers lacking partial agonist activity has also been reported to be invariably associated with a reduction on CO (28). Our data is at contrast, with these reports. The reduction in CO after administration of AC and MET was associated with a return of the initially reduced CO to normal values as we did not observe any significant differences between placebo, MET and AC after chronic administration (Fig 7-3). The fact that CO is restored to an initially depressed cardiac function after administration of these two agents implies that this is probably independent of the effect of ISA possessed by AC. Nevertheless, this is in agreement with other reports which found the restoration of CO occurs after the administration of timolol and atenolol, two β-blockers, devoid of ISA (39,40). Furthermore, the restoration of CO after long term administration has also been confirmed by comparing the long term administration of atenolol and propranolol, β-blockers devoid of ISA, with AC, a β-blocker which possesses ISA (41).

In parallel with the CO, the RBF is not affected after chronic administration of either AC or MET. We did not find any significant differences in the liver, kidneys, heart, spleen, brain or stomach blood flow after chronic administration of either drug as it compared with the control values. Van den Meiracker *et al* have reported that after 4-6 weeks treatment with AC they did not find any significant differences in renal blood flow between the AC and control treatment in hypertensive subjects (41). Other investigators also could not detect any significant differences in organ blood flow after chronic administration of AC as they measured this values in the muscle, calf, or kidneys (35,38,42). Similar results have also been reported after long term administration of MET and measuring of calf (43) and skin blood flow (7).

The principle of this study was to evaluate the hemodynamic differences and in particular the differences which may occur in CO and RBF after administration of a β blocker which is devoid of ISA with a β-blocker which possesses ISA. Our results do not quantify any differences between these two agents as the inter-individual variations preclude any conclusion from this study about differences between the drugs. However, an interesting hemodynamic change during this study was noticed. The acute reduction in CO and RBF was observed at stage one. This CO and RBF reduction was associated by a correction of CO and RBF toward the baseline values at the second stage. The changes in CO and RBF at this stage were not statistically significant as compared with the control, however these values decreased and fell below the baseline pretreatment values. In the last stage, the CO and RBF were less than pretreatment baseline values but the differences were not statistically significant. These finding can be interpreted as being consistent with the hypothesis of autoregulation (44). Auto-regulation, or in another words, rapid local control of tissue blood flow, has been characterized in almost every organ system or body. This term explains that either over or underperfusion of an isolated tissue bed, brought about by raising or lowering the regional blood perfusion pressure, results in local adjustment of vascular resistance which returns blood flow toward normal level. Two types of autoregulation have been described, short term and long term. The first one appears within hours of over or underperfusion and the latter appears within days or months. Observations on our study show that at the second stage the CO and RBF return toward normal as these values are not significantly different as they compare with the control. This restoration of CO and RBF will stay constant after chronic administration as we observed in the third stage of our study. Return of these values to a pretreatment baseline may be consistent with the long term auto-regulation as it has been discussed elsewhere (44). There has been controversy regarding the role of autoregulatory mechanisms in short or long term blood pressure control (39,45,46) and the relation between these two events. Our observations do not allow assessment of mechanisms which are involved in this regard. It only adds a new set of observations to this debate.

In conclusion, the present study demonstrates that AC and MET significantly reduced the CO and RBF in most organs after acute administration in rat as compared with the baseline values. However, these values returned to normal after chronic administration of either AC or MET. Thus, our results suggest that the additional property of ISA possessed by AC result in no statistically significant hemodynamic differences from MET. This may reflect an insufficient degree of ISA possessed by AC. The considerable inter-individual variability in this study may also explain the difficulty to inference any significant differences between these two agents.

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Summary and Conclusions

In this research the pharmacokinetics and selected pharmacodynamics of AC and MET were evaluated.

The rat appears to be a good animal model to study the pharmacokinetics of AC as the disposition characteristics of AC enantiomers were comparable to their disposition in human. The plasma concentrations of AC obtained after iv administration of 5 mg kg^{-1} and 15 mg kg^{-1} dose of AC in rat were in close range to those seen in human after iv doses of 0.4 and 1.2 mg kg^{-1} (1). Furthermore, the absorption of AC enantiomers in rat were very similar to that in human, since in both species two concentration maxima were observed in the concentration *vs* time profiles of AC enantiomers. Following iv and oral administration of AC to rats, the disposition of the AC enantiomers were found to be stereoselective in that the CL_T of S-AC was greater than that of R-AC.

Dose dependent pharmacokinetics have been reported for numerous drugs including beta-blockers. Moreover, the use of AC in hypertension, angina pectoris or arrhythmias requires different doses. This may result in differing pharmacokinetic profiles of AC and DC enantiomers. Nonetheless, for the further studies on AC, a suitable dose had to be chosen. Thus, we studied the dose ranging comparisons of AC enantiomers in rats. The results obtained after administration of 5 to 50 mg kg^{-1} of AC indicated that the disposition of AC enantiomers is linear over the dose range studied. The CL_T, V_d, $t_{1/2}$ or urinary recovery and CL_R of AC enantiomers were all linearly related to dose ($p < 0.05$). When the observed plasma concentrations were normalized to the doses administered, the observed concentration-time profiles following the four doses were virtually superimposable. On the other hand, a plot of the AUC *versus* dose was linearly related for AC in the rat over the dose range studied for both R- ($r = 0.94$, $P<0.05$) and S- ($r = 0.95$, $P<0.05$) enantiomers.

It was observed that AC enantiomers accumulated after multiple oral doses of the racemate. This might be due to either an increase in bioavailability or a decrease in hepatic clearance of AC. The bioavailabilities of the AC enantiomers were about 34% and 36% after a single dose administration for R- and S-AC, respectively. These values were increased to 51% and 53% after multiple doses of racemate. Furthermore, P.O. bioavailability is a function of pre-systemic extraction by enzymatic metabolism primarily on first liver passage. The bioavailability of AC is low because of this first pass as it has been reported that after P.O. administration, AC is almost completely absorbed (2). Therefore, saturation of first-pass metabolism appears the most likely explanation for the increase in bioavailability of AC observed after multiple dosing.

Interestingly, we found that the ratio of DC to parent drug recovered in the urine was lower after multiple P.O. dosing compared with the single dose data. This may suggest that the CL_{int} is reduced after multiple dosing. A decrease in intrinsic hepatic clearance would be expected to increase bioavailability at steady state with little or no change in systemic clearance. This is consistent with the lack of changes in β at steady state compared with those after single dose.

Another possible explanation for a change in AUC after multiple dosing is a possibility of a decrease in blood flow due to this multiple dosing. A change in hepatic blood flow may have a significant effects on hepatic clearance of AC, as we found that AC is a moderately extracted drug in rat. If this is the case, blood flow should also be decreased after intravenous administration, resulting in drug accumulation after multiple dosing. This possibility was tested and no significant differences were observed between the $AUC_{0-\infty}$ of AC enantiomers after single iv dose as compared with $AUC_{0-\tau}$ after multiple iv doses of the racemate. In other words, the time courses of AC enantiomers in plasma were virtually superimposable. Therefore, it can be concluded that the increase in bioavailability after multiple oral dosing is not due to a change in systemic clearance of AC enantiomers due to a decrease in hepatic blood flow.

The interesting point observed after P.O. administration of AC was the existence of multiple peaks in the absorptive phase of our rats. This was observed after both single or multiple administration of AC. Different mechanisms have been suggested for multiple peaking including enterohepatic recycling (3), discontinuous gastrointestinal absorption (4-6), formation of poorly absorbed micelle complex of drug with bile salts (7,8), variable gastric emptying (9,10), gastric pH (11), or reversible metabolism (12). As the multiple peaks phenomenon was not observed after iv administration of AC it is unlikely that enterohepatic re-circulation plays an important role in the appearance of double peaks after P.O. dosing. Furthermore, as the rat had been shown to be a suitable model for evaluating the double peak phenomena of AC it was of interest to explore the reason for this phenomena (13). Therefore, various hypotheses were examined.

The hypothesis that gastric pH plays an important role in the generation of double peaks following oral administration of AC was evaluated by co-administration of CIM as a method to increase intragastric pH. Concomitant CIM administration did not change the concentration-time profiles of AC enantiomers. In fact, CIM was co-administered to increase the intragastric pH, as it has been shown that CIM increases the gastric pH which in turn increases the gastric emptying rate (11) resulting in disappearance of double peaks in the plasma profiles of AC enantiomers. With AC however, the double peaking was retained after CIM administration.

Another plausible explanation for multiple absorption peaks in concentration time profiles of AC enantiomers involves the formation of poorly absorbed micelle complexes of drug with bile salts. This is a reasonable expectation as AC is reportedly excreted via the bile. We have also tested this hypothesis to see whether this could cause two peaks after oral administration of AC. Following oral administration of AC to a bile duct cannulated rat the double peak phenomena remained.

Various models of discontinuous gastrointestinal absorption have been developed to describe the multiple peaks seen after oral administration of drugs (14-16). Among those models, the existence of a classical absorption window which is incorporated with a non-absorbing gastrointestinal segment between one or more absorption sites is the more probable model which may explain our observations. The absorption window concepts was tested by administration of AC to different segments of intestine in a conscious rat. It was found that duodenal administration of AC had no effect on double peaks in the plasma concentration time profile, which also confirms our previous finding that gastric emptying has no effect on generating double peaks. Instead, the double peaks disappeared following ileal administration of AC, confirming the hypothesis that absorption windows play an important role in generating the double peaks in plasma profiles of AC enantiomers.

The accumulation of drug after multiple administration was more apparent with MET. A significant increase in plasma profiles of both R- and S-MET was found after oral administration of racemate as compared to single dose data. This increase in AUC may be due to either a decrease in clearance or an increase in bioavailability due to multiple dosing. Nonetheless, the marked effects of multiple dosing on bioavailability of MET has been reported by others. Furthermore, we found that the disposition of MET in rats was also stereoselective after oral administration of racemate.

Finally, although we found that AC and MET accumulated in the body after multiple dosing, it was not known whether this accumulation was due to a change in blood flow after multiple dosing. We therefore studied the influence of these two β-blockers on blood flow. A reduction of hemodynamic parameters by both AC (with ISA) and MET (without ISA) in hypertensive patients has been shown (18). A survey of the literature, however, indicates that drugs with ISA give less of a reduction in resting and maximal exercising heart rate and consequently in CO, than those without ISA. This evidence also suggests that peripheral resistance and peripheral blood flow is less reduced by the ISA effect. Thus the presence of ISA may have a crucial effect on hemodynamic parameters. It was therefore proposed to study in detail the pharmacodynamics of these β-blockers to assess the impact of ISA in this regard.

A rapid fall in CO, observed after acute administration of AC and MET largely agrees with previously reported responses to these drugs when administered intravenously (17). The differences in percent of change in CO between these two agents were not statistically significant ($p < 0.05$), however, a trend towards an increase in this value was observed after MET administration. As AC has a relatively low degree of ISA among β-blockers it may not have enough sympathomimetic activity to induce significant difference between this drug and MET (which has no ISA). Nonetheless, other investigators have also failed to detect any significant differences in hemodynamic changes caused by β-blockers with relatively low degrees of ISA as they have compared them with the β-blockers which are devoid of ISA (17). Moreover, Manttari *et al.* reported that the hemodynamic differences between MET and AC are not statistically significant in hypertensive patients (18).

The change in RBF after acute administration of either AC or MET was significant when the blood flow was compared with the baseline values. The percent of change in RBF however, is significant in the liver, heart and spleen only one minute after drug administration as compared with the same values in the control group.

Following long term administration of either AC or MET the CO remains unchanged. The fact that CO is restored after an initially depressed cardiac function after administration of these two agents implies that this is probably independent of the

ISA effect. Nevertheless, this is in agreement with other reports that found the restoration of CO occurs after the administration of timolol and atenolol, two β-blockers, devoid of ISA (19,20). Furthermore, the restoration of CO after long term administration has also been confirmed by comparing the long term administration of atenolol and propranolol, β-blockers devoid of ISA, with AC, a β-blocker which possesses ISA (21).

In parallel with the CO, the RBF is not affected after chronic administration of either AC or MET. We did not find any significant differences in the liver, kidneys, heart, spleen, brain or stomach blood flow after chronic administration of either drug as compared with the control values. Other investigators have not detected any significant differences in organ blood flow after chronic administration of AC as they measured these values in the muscle, calf, or kidneys (21-24). Similar results have also been reported after long term administration of MET and measuring of calf (25) and skin blood flow (26).

In summary, these studies have found that 1) Sprague-Dawley rat appears to be a good animal model for studying the pharmacokinetics of AC and MET; 2) AC and MET dispositions are stereoselective in the rat model after administration of racemates; 3) the pharmacokinetics of AC enantiomers is linear in therapeutic doses in a rat model; 4) AC is a moderately extracted drug in rats; 5) the window of absorption is the underlying mechanism for the appearance of multiple peaking in concentration time profiles of AC enantiomers; 6) the pharmacokinetics of AC and MET enantiomers at steady state could not be predicted after single oral dose administration of these agents; 7) AC and MET decrease the CO and RBF after acute administration but these values return to normal after chronic administration and; 8) there are no significant difference between AC and MET with regard to hemodynamic changes caused by these two β-blockers.

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